University of Alberta

Assessment of Iron Status in Adolescents: Dietary, Lifestyle and Biochemical Determinants

by

Heather E. Deegan ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment
of the requirements for the degree of Master of Science

in

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ABSTRACT

Impaired iron status can have detrimental physiological outcomes. The objective of the study was to determine the prevalence of iron deficiency in adolescents (*n*=396) and assess the occurrence of potentially influential factors. It was hypothesized that low dietary iron intakes and behavioural and medical factors would be associated with impaired iron status. Iron status, iron intake, dieting, vegetarianism, recent blood donation, intense exercise, vitamin/mineral supplement use, smoking cigarettes, recent infection, previous diagnosis of anemia, and heavy menstrual flow and number of years since menarche (among females) were measured. The prevalence of iron deficiency among adolescent females and males was 6% and 0%, respectively (female *n*=232). Dietary iron intake was the only factor that was different between iron compromised and iron replete females. Although mean iron intake was lower among iron deficient girls (p=0.001), mean iron intakes in both the iron deficient and iron replete groups exceeded the RNI. Overall, the results suggest that the adolescents surveyed are at low risk for iron deficiency relative to other similar populations. Future studies should assess the type and bioavailability of dietary iron, as this may be more important than total iron intake in relation to iron status.

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ABBREVIATIONS

BMI:	Body mass index (kg/m^2)
FAO:	Food and Agriculture Organization
FFQ:	Food frequency questionnaire
kcal:	Kilocalories
MCHC:	Mean cell hemoglobin concentration
MCV:	Mean cell volume
NHANES:	National Health and Nutrition Examination Survey
RNI:	Recommended nutrient intake
SFFQ:	Semi-quantitative food frequency questionnaire
TIBC:	Total iron binding capacity
UIBC:	Unsaturated iron binding capacity
WHO:	World Health Organization

DEFINITIONS

Dieter

For the purposes of the present study, a participant was considered a dieter if they indicated they were currently eating less than usual to try and lose or maintain weight.

Lacto vegetarian

For the purposes of the present study, a participant was considered a lacto vegetarian if they eliminated animal flesh and eggs from their diet, but included milk and milk products.

Lacto-ovo vegetarian

For the purposes of the present study, a participant was considered a lacto-ovo vegetarian if they eliminated animal flesh from their diet, but included eggs, milk and milk products.

Inadequate dietary iron intake

In the present study, dietary iron intake was considered "low" or "inadequate" if it was less than 2/3 of the age and gender specific Recommended Nutrient Intake (RNI).

Definition adapted from Gibson, 1990b

Iron deficiency

Iron deficiency is an advanced stage of iron depletion that results from a state of negative iron balance. Iron deficiency indicates that iron stores have been depleted and the functional iron pool has been affected.

Definition adapted from the Expert Scientific Working Group, 1985

Iron deficiency anemia

Iron deficiency anemia results from a prolonged state of negative iron balance and is the final stage of iron depletion. The production of iron-containing functional compounds is impaired and red blood cells shrink and lose their characteristic red color.

Definition adapted from Herbert, 1992

Ovo vegetarian

For the purposes of the present study, a participant was considered an ovo vegetarian if they eliminated animal flesh, milk and milk products from their diet, but included eggs.

Relative validity

Validity is the degree to which a dietary assessment instrument measures what it intends to measure.

Relative validity is a measure of the validity of a dietary intake method. It evaluates a "test" diet assessment method against a "reference" method. The validity is considered "relative" because the absolute truth, or true validity, of dietary intake is difficult to measure.

Definition adapted from Gibson, 1990b

Storage Iron depletion

Storage iron depletion is the mildest state of iron deficiency and is detectable when serum ferritin has dropped below normal. Functional iron may not be affected in this early stage and there usually are no physiological impairments.

Definition adapted from Herbert, 1992, Bothwell et al., 1979

Vegan vegetarian

For the purposes of the present study, a participant was considered a vegan if they eliminated all foods of animal origin (red meat, poultry, fish, milk and milk products and eggs) from their diet.

Vegetarian

For the purposes of this study, a vegetarian was defined as an individual who excluded meat and eggs, excluded meat, excluded meat, milk and milk products, or excluded meat, milk and milk products and eggs from their diet (i.e. "vegetarian" encompassed all previous definitions of vegetarian and vegan vegetarians).

CHAPTER ONE
INTRODUCTION

A. RATIONALE

Iron deficiency remains the most common nutritional deficiency worldwide (Centers for Disease Control and Prevention, 1998). Despite an overall decrease in the global prevalence of iron deficiency due to fortification programs, the prevalence of iron deficiency remains high and is currently estimated at 9-11% in American women of childbearing age (Looker et al., 1997). Estimates are higher among Canadian women. A study conducted in 1985 by Seoane and colleagues estimated 35% of their female population was iron deficient. Women in particular are at increased risk for iron deficiency as menstruation elevates the physiological requirement for this essential micronutrient. Several factors can lead to the development of iron deficiency in the normal physiological state, including blood loss, inadequate dietary intakes of iron, limited absorption of dietary iron, rapid growth, and heavy menstrual flow in females (Centers for Disease Control and Prevention, 1998). Some of the outcomes of iron deficiency include developmental delays and behavioural disturbances in children, anorexia, compromised immune function, impaired work capacity or athletic performance as well as decreased ability to concentrate (Centers for Disease Control and Prevention, 1998).

The adolescent population is a group that is at particularly high risk for the development of nutritional disorders, especially iron deficiency due, in part, to the physiological demands of growth. Among teenage girls, the concomitant occurrence of menarche and subsequent regular menstruation surrounding the growth spurt imposes a regular challenge to maintain positive iron balance. Several other issues, unique to the adolescent population, may present additional challenges to establishing and maintaining a healthy iron nutriture, including food choices and lifestyle behaviours.

Although there is stringent tracking of national food consumption patterns and nutritional status in the U.S., recent similar data is lacking in Canada, especially in the adolescent population. Research is needed to determine the iron intake of adolescents in

Canada and the factors that influence iron nutriture.

B. PURPOSE

The current research was conducted to assess the dietary intake and iron status of an adolescent population in Edmonton and to determine the factors that may influence iron status. A group of apparently healthy, middle-class female and male adolescents was assessed to determine iron status, dietary intake, dietary behaviours, lifestyle behaviours and medical history.

Adolescents constitute a group with unique health concerns and sometimes peculiar diet and lifestyle behaviours. The amount of scientific research addressing the health and nutritional issues of adolescents is minimal in Canada. By assessing the diet and lifestyle habits and iron health of adolescents, researchers and health care professionals may further understand the behaviours, perceptions, cognitions and specific nutritional needs of this population. This information may be useful in identifying specific health concerns within the adolescent population and may assist in the development of educational strategies to improve the overall health of Canadian teenagers.

C. HYPOTHESES

The research hypotheses for this study were as follows:

1. Adolescents with low dietary iron intakes (less than 2/3 of the RNI) will have iron deficiency or iron deficiency anemia.
2. Adolescents with low dietary iron intakes (less than 2/3 of the RNI) will be either dieting for the purposes of weight loss or weight maintenance or will be following a vegetarian diet.
3. Behavioural factors such as recent blood donation, intense exercise, vitamin/mineral supplement use and smoking cigarettes will be predictive of iron status in adolescents.
4. Medical factors such as recent infection, previous diagnosis of anemia, heavy menstrual flow and number of years since menarche in females will be predictive of iron status in adolescents.

D. OBJECTIVES

The objectives of the present study were:

1. To measure the iron status of adolescents by assessing a blood sample for serum ferritin, transferrin saturation and hemoglobin concentration.
2. To determine the presence of iron deficiency or iron deficiency anemia in adolescents using age- and gender-specific cutoff values and a multiple criteria method for iron status determination.
3. To determine the prevalence of iron deficiency and iron deficiency anemia in the adolescent population.
4. To assess the usual daily dietary intake of iron, energy and macronutrients among adolescents using a food frequency questionnaire.
5. To measure the relative validity of the food frequency questionnaire in the adolescent population using a three-day estimated diet intake record in a sub-sample of the larger study population.
6. To develop and utilize a questionnaire to assess the diet behaviours, lifestyle behaviours and medical factors related to the iron status of adolescents and determine the influence of these factors on iron nutriture.

CHAPTER TWO
LITERATURE REVIEW

Iron nutrition and the multiple factors that influence iron metabolism, function, and storage has been extensively researched and is well understood. Despite this vast body of research, several population groups remain at risk for compromised iron status. Adolescents, especially adolescent females, comprise a group that is identified as "high risk" for poor nutrition and sub-optimal iron status (Centers for Disease Control and Prevention, 1998). The physiological importance of iron and the methods for determining iron status and nutritional intake will be explored through the present research, as well as related dietary, behavioural and medical factors unique to adolescents. Currently, the research available exploring nutrient intakes and iron status of Canadian adolescents is limited.

A. IRON PHYSIOLOGY

Iron deficiency is the most frequently observed nutrient deficiency in the world (Looker et al., 1997; DeMaeyer, 1989). There are no current estimates for the prevalence of iron deficiency in Canada. However, recent estimates from the United States based on the third National Health and Nutrition Examination Survey (NHANES III, 1988-1994) indicate that young children, adolescent females, women of reproductive age and the frail elderly are at the highest risk for iron deficiency (Looker et al., 1997). In absolute numbers, this translates to 7.8 million adolescent girls and women of childbearing age with iron deficiency and 3.3 million with iron deficiency anemia in the United States (Looker et al., 1997). Clearly, these conditions remain an important health concern in vulnerable populations at both national and international levels and cannot be overlooked.

1. Function of Iron

Iron is present in all cells of the body and serves many vital functions. Iron is a carrier for oxygen and is important for oxygen utilization and storage. Electron transport and several enzyme reactions also depend on iron for optimal functioning. Without adequate amounts of this element, morbidity and mortality ensue.

Iron is unique in that it can readily change from its oxidized state, ferric iron (Fe^{3+}), to its reduced state, ferrous iron (Fe^{2+}). This property enables iron to donate and accept electrons and hence, act as a catalyst in many redox reactions. This oxidative, and potentially harmful property of iron is controlled by bound proteins or the presence of antioxidant molecules (Yip & Dallman, 1996).

Iron exists in the body in two compartments: functional iron and storage iron. When iron intake and absorption are sufficient, most iron (>70%) is found in the functional form (Centers for Disease Control and Prevention, 1998). The majority of functional iron is present as heme protein. Heme is widely distributed in hemoglobin within red blood cells where it functions to accept and release oxygen for transport. Heme is also found in myoglobin within muscle cells where it facilitates oxygen use and storage. Some additional functional iron is found in enzymes, such as cytochromes. Cytochromes contain heme and are essential for mitochondrial electron transport, and thus respiration and energy metabolism (Yip & Dallman, 1996). Hepatic catabolism of chemicals and toxins also depends on cytochromes. Other iron-containing enzymes play a role in neurotransmitter systems in the brain and in amino acid metabolism, both of which maintain normal brain function. Enzyme iron is also required for leukocyte function, the neutralization of highly reactive free radicals and the synthesis of steroid hormones and bile acids. Iron-containing enzymes also function as co-factors in numerous other essential physiological activities. Despite the essential nature of these substances, the iron contained in enzymes comprises only 3% of total body iron (Yip & Dallman, 1996).

When body levels of iron exceed the amount required for functional purposes, it is stored as a soluble protein complex, ferritin, or as an insoluble protein complex, hemosiderin (Bothwell, 1995). This storage form of iron is found in the spleen, bone marrow, liver and skeletal muscle, and to a lesser extent in the plasma. In healthy individuals, iron is primarily stored as ferritin (70-80%). This reservoir is utilized for cellular iron requirements, the majority of which involve the manufacture of red blood cells. Approximately 80% of body iron is used for erythropoiesis and hemoglobin production (Yip & Dallman, 1996).

A small amount (<1%) of iron is found in the plasma bound to a protein complex called transferrin. Iron from the intestine or from the breakdown of hemoglobin binds to transferrin and is delivered to tissues that possess a high requirement for iron, such as bone marrow, where iron is required for erythrocyte production (Yip & Dallman, 1996). The amount of iron available to tissues is reflected by the saturation of iron receptors on the transferrin molecule. Nutritional iron deficiency occurs when there is insufficient iron to meet the demands for functional iron after stores have been exhausted. Iron deficiency can also occur when the release of storage iron is impaired, despite adequate intake and stores.

2. Metabolism of Iron

Iron balance and metabolism are affected by three main factors: intake, storage and loss. The uptake of iron at the gastrointestinal tract is determined by the bioavailability and quantity of iron in the diet and by the body's potential to absorb iron. Iron storage and iron loss are influenced primarily by the body's current iron status and physiological requirement, however there are several other influential factors.

2.1 Iron intake and absorption

The primary regulatory mechanism of iron balance is absorption at the intestinal mucosa. The amount absorbed from food can vary from <1% to >50%, depending on the body's physiological need for iron, the amount of iron in the body, the availability of body iron for cellular functions and the rate of red blood cell production. Iron absorption also depends on mucosal regulatory mechanisms, the amount and type of iron ingested and the presence of absorptive enhancers and inhibitors in the diet (Skikne & Baynes, 1994). Under ideal conditions, an individual maintains functional iron and will accumulate iron stores. The main factor controlling iron absorption is the amount of storage iron. When stores are adequate, the absorptive mechanisms in the gut will be down-regulated and when stores are low, absorption will be up-regulated. Iron uptake is also affected by increases in erythropoesis (Bothwell, 1995; Skikne & Baynes, 1994).

Females generally absorb iron more efficiently than males. This reflects an increased physiological requirement due to lower iron stores as a consequence of menstruation. In a state of iron deficiency, absorption increases proportionately (Finch, 1984; Hallberg, 1981). Pregnancy also elicits an increase in iron absorption to support the expanding blood volume and the iron requirements of the growing fetus. This high level of absorption is temporary and decreases post-partum, reflecting an accumulation of maternal iron stores.

Iron absorption can occur at any location along the small intestine, but appears to be most efficient at the duodenum (Fairbanks, 1994). The type of dietary iron and its subsequent bioavailability is a large determinant of the amount of iron that can be absorbed. The iron in food exists in two forms; heme iron and nonheme iron. The two forms of iron have different pathways of uptake at the intestinal mucosa. Despite extensive investigation, the features and mechanisms of intestinal iron absorption are still not clearly understood (Latunde-Dada et al., 1998).

Heme iron is absorbed intact and is processed within mucosal cells (Finch & Cook, 1984). The protein portion of the ingested heme, in the form of hemoglobin or myoglobin, is either removed by proteolytic enzymes in the duodenum or removed within the mucosal epithelium. Once inside the epithelial cells, iron is liberated from heme and is then transferred to the plasma (Fairbanks, 1994). Heme iron is found only in fish, poultry and meat. Although it accounts for a relatively small proportion of the diet, heme iron is absorbed two to three times better than nonheme iron (Zeigler, Filer, 1996; Carpenter & Mahoney, 1992). Heme iron is absorbed very well and is not influenced by dietary factors to any appreciable extent, with the exception of calcium. Evidence from Hallberg and colleagues (1991) indicates that heme iron absorption is decreased by calcium from milk, cheese and calcium chloride. The absorption of heme iron is increased slightly in the state of iron deficiency (Fairbanks, 1994).

The bioavailability of nonheme iron is strongly influenced by the composition of the meal in which it is ingested (Dwyer, 1991). Nonheme iron is composed of iron salts and comprises the majority of iron in the diet, usually >85% (Fairbanks, 1994; Carpenter & Mahoney, 1992). Plant foods, dairy products and iron-fortified foods are the primary dietary sources of nonheme iron (Fairbanks, 1994; Hallberg, 1981). Dietary constituents impact nonheme iron absorption largely by affecting its solubility in the alkaline environment of the small intestine. Several dietary factors decrease nonheme absorption. These inhibitors include phytates (soy, bran, cereal, nuts, legumes, wheat, rice), polyphenols (vegetables, tea, legumes), phosphates (legumes), milk and soy proteins, albumin, calcium (dairy products), tannins (tea), coffee, antacid preparations and zinc salts. In addition, the basic environment of the gastrointestinal tract can cause alkalinization and subsequent precipitation of nonheme iron (Yip & Dallman, 1996; Bothwell, 1995; Fairbanks, 1994; Disler et al., 1975).

The inhibitory effects of the above dietary constituents can be decreased by factors that enhance nonheme iron absorption. The most powerful promoter is ascorbic acid, the effects of which appear to be dose-related (Siegenberg et al., 1991). Despite its positive effects on nonheme absorption, the extent of the influence of vitamin C depends on the interaction between it and the inhibitory factors present in the ingested meal (Bothwell, 1995).

In addition to ascorbic acid, factors present in meat also enhance the absorption of nonheme iron. It is unclear whether the enhancing property can be attributed to the heme iron itself, polypeptides contained in meat, fish and poultry, or the products of meat digestion. Regardless, meat is beneficial for iron nutrition in two ways; it is a highly bioavailable source of iron and it enhances the uptake of nonheme iron at the intestinal mucosa. The inclusion of modest amounts of beef in the diet results in a twofold increase in apparent iron absorption and utilization (Johnson & Walker, 1992). These benefits are associated specifically with meat protein, not with animal proteins in general (Bothwell, 1995; Cook & Monsen, 1976).

Similar to heme iron, nonheme iron absorption is enhanced in the state of iron deficiency. However, the increase in absorption rate is more pronounced for nonheme iron, and may elevate to 50% in severe iron deficiency (Hallberg, 1981). Periods of rapid growth throughout the lifecycle, such as infancy and childhood, adolescence and pregnancy, also induce a higher rate of nonheme iron absorption.

2.2 Iron transport and storage

Once iron has traversed the intestinal epithelium or has survived the life span of a red blood cell, it is picked up in the plasma by transferrin, an iron carrier. This transport protein retrieves iron from blood or lymph after absorption or from macrophages after hemoglobin degradation. The distribution and delivery of iron throughout the body depends on cellular demand, although erythrocyte precursors in the bone marrow receive most of the iron for hemoglobin synthesis (Fairbanks, 1994). When all iron binding sites on the transferrin molecule are occupied, it is saturated. High saturation of transferrin indicates an oversupply, while low saturation indicates an undersupply, or deficiency, of iron. When transferrin is highly saturated, absorbed iron cannot be bound for transport and is in excess of body requirements.

Excess iron is deposited in the cells of the liver, spleen and bone marrow (Fairbanks, 1994). Iron is stored intracellularly as ferritin and hemosiderin. This reservoir of stored iron supplies cells that have a high demand, such as erythrocytes. Under normal conditions, there appears to be a propensity for iron to be stored as ferritin over hemosiderin (Fairbanks, 1994). Ferritin is soluble in aqueous media, whereas hemosiderin is not. Hence, the iron bound to ferritin is more readily available to obligatory tissues. Although there are other subtle differences at the molecular level, both forms can be mobilized as the need for iron increases.

The effectiveness of the body's regulatory mechanisms for protecting it against excess iron is not known (Bothwell, 1995). In positive iron balance, over the long term, the storage compartment of iron within the body will continue to grow, despite a low rate of absorption of dietary iron. This may be problematic because, while iron fortification programs are directed at vulnerable groups, iron fortified foods are also frequently consumed by segments of the population who are not at risk for compromised iron status, such as adult men. Evidence regarding the long-term effects of iron fortification on iron-replete individuals is inconclusive (Bothwell, 1995).

2.3 Iron reutilization and loss

Three unique mechanisms maintain iron balance and prevent iron deficiency. The first is the regulation of absorption at the intestinal mucosa, as mentioned above. The second is the access to storage proteins (ferritin and hemosiderin) to meet iron demands in excess of absorptive potential, also mentioned above. The third mechanism is the continuous reutilization of iron from the catabolism of red blood cells. The main source of recycled iron is from the degradation and production of erythrocyte cells. A normal red blood cell is catabolized after approximately 120 days. Iron from the heme portion of degraded erythrocytes is recaptured for the synthesis of hemoglobin and new red blood cells (Yip & Dallman, 1996). Transferrin molecules are responsible for recapturing and delivering iron to red cell precursors in the bone marrow or other cells in tissues undergoing growth and development (Hallberg, 1992). Greater than 90% of the iron contained in hemoglobin is continuously recycled (Fairbanks, 1994). Iron that cannot be immediately incorporated into functional cells enters the storage compartment as ferritin or hemosiderin (Yip & Dallman, 1996).

Despite this mechanism for prudent iron conservation, some loss (0.6 mg/day) occurs through desquamated mucosal cells, bile and blood in the feces (Yip & Dallman, 1996). Smaller amounts of iron are lost through sloughed skin cells, sweat, urine and respiration. Regular iron loss in healthy individuals is limited and increases only slightly in states of iron overload (Fairbanks, 1994). The iron lost in menstrual blood of premenopausal women cannot be recovered and accounts for the higher iron requirements in this population. Additional blood and iron loss can occur as a result of gastrointestinal bleeding from peptic ulcer disease, inflammatory bowel syndrome, bowel cancer and cow's milk sensitivity in infants and children (Centers for Disease Control and Prevention, 1998).

3. Measurement of Iron Status

There are several tests available to assess iron status. They include serum ferritin, transferrin saturation, hemoglobin concentration, serum iron concentration, total iron binding capacity (TIBC), serum transferrin, serum transferrin receptors, hematocrit, mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), free erythrocyte protoporphyrin concentration, and red blood cell distribution width. Some of these tests measure iron supply for erythroid synthesis while others assess body iron stores. No single iron parameter monitors the entire spectrum of iron status. For this reason, a multiple variable model is the accepted method for detecting iron deficiency in a population. The use of a combination of measurements increases the specificity of the diagnosis of anemia (Hallberg, 1992). In addition, it avoids the misclassification that can occur with a single indicator method (Looker et al., 1995, Gibson, 1990a). Only the measures of iron nutriture utilized in the present research study will be discussed in detail.

3.1 Serum ferritin

Serum ferritin is the preferred measure of body iron stores and indicates early changes in iron status (iron depletion) (Beaton et al., 1989). Serum ferritin is the only parameter that is affected at the earliest and least severe stage of iron deficiency (Cook & Finch, 1979). It is the most sensitive test of iron deficiency and the most specific indicator of depleted iron stores, especially when used together with other tests of iron status (Ahluwalia, 1998; Centers for Disease Control and Prevention, 1998). Under normal conditions, the amount of serum ferritin directly correlates with the amount of stored iron in the body at any given time (Bothwell et al., 1979a; Cook & Finch, 1979; Lipschitz et al., 1974). A drop in serum ferritin is augmented by a decrease in iron stores. Ferritin enables differentiation between iron deficiency anemia and the anemia of chronic infection, whereas other measures are similarly influenced by these two conditions (Cook & Finch, 1979).

Although serum ferritin is a relatively accurate index, it can become elevated in inflammation, hyperthyroidism, mild and/or chronic infection and in diseases that cause organ or tissue damage, independent of iron status (Hallberg, 1995; Lipschits et al., 1974). Oral contraceptive use also causes serum ferritin to increase (Tietz, 1990a). In these cases, depleted iron stores would not be detected. Since there is no laboratory test to indicate individuals free from infection (Hallberg, 1995), it is important to account for this confounding variable in prevalence studies relying on ferritin for detecting iron deficiency.

3.2 Transferrin saturation

Transferrin saturation is a calculated value that denotes the supply of iron to tissues. When transferrin saturation is low, there is a high proportion of available iron binding sites. This reflects a limited availability of iron for transport. The calculated value for transferrin saturation is based on two biochemical measures, TIBC and serum iron concentration. Serum iron concentration is divided by TIBC and multiplied by 100. The result is expressed as a percent.

$$\text{transferrin saturation (\%)} = \frac{\text{serum iron concentration } (\mu g/dL)}{\text{total iron binding capacity } (\mu g/dL)} \times 100$$

TIBC is an indicator of the capacity of iron to bind in the serum and reflects the vacancy of iron binding sites on the transferrin molecule, and hence, its iron carrying capacity (Gibson, 1990a). Transferrin is considered "saturated" when all iron binding sites are occupied. When serum iron concentration (and stored iron) is high, TIBC is decreased and when serum iron concentration (and stored iron) is low, TIBC is elevated. Many factors can affect the results of this measure, including chronic infection, inflammation, malignancies, liver and kidney disease and malnutrition (Fairbanks, 1994). Oral contraceptive use and pregnancy can elevate this test result (Looker et al., 1995; Beaton et al., 1989). Changes in TIBC occur only after iron stores are depleted, making it a less sensitive index of iron deficiency (Fairbanks, 1994).

Serum iron concentration measures the total number of iron atoms bound to transferrin (Gibson, 1990a). Serum iron decreases in iron deficiency and increases in other types of anemia (Tietz, 1990b). This measure is influenced by food consumption, in that it shows a post-prandial increase. In addition, it is decreased in the presence of infection or inflammation (Tietz, 1990b; Yip & Dallman, 1988). Serum iron concentration also demonstrates inconsistent diurnal variation which varies for an individual within the same day and from day-to-day (Tietz, 1990b; Beaton et al., 1989).

Since transferrin saturation is a calculated measure based on the above two indices, it is also subject to the same factors that affect TIBC and serum iron concentration. Although not as sensitive to changes in iron stores when compared to serum ferritin, transferrin saturation is a good indicator of iron deficient erythropoesis (Herbert, 1992). For this reason, when used in combination with serum ferritin, transferrin saturation is an effective method for determining iron nutritional status and the stage of iron deficiency.

3.3 Hemoglobin concentration

Hemoglobin concentration is a common laboratory test that provides a direct and sensitive measure of functional iron in the body (Centers for Disease Control and Prevention, 1998). Hemoglobin is considered a late indicator of iron deficiency as it does not decrease until advanced depletion of body iron. However, this index is essential for the determination of iron deficiency anemia (Centers for Disease Control and Prevention, 1998). The measure of hemoglobin concentration does not indicate the cause of anemia, but when combined with other measures of iron status it can be used to differentiate iron deficiency anemia from other types of anemia (Centers for Disease Control and Prevention, 1998). Anemia can be caused by factors other than iron deficiency, including vitamin B_{12} or folate deficiency, hereditary influences on red blood cell production, recent or current infection and chronic inflammation.

Hemoglobin concentration is subject to small diurnal variation, however these are minor influences and are considered insignificant from both a biological and statistical perspective (Borel et al., 1991; Beaton et al., 1989). The utilization of hemoglobin to estimate the prevalence of iron deficiency anemia is also subject to ethnic differences. Subsequently, different hemoglobin cutoffs have been established for some ethnic groups. For example, appropriate cutoff values have been established for adolescent girls in Jamaica, since individuals with African heritage have lower hemoglobin values compared to Caucasian girls with similar iron status (Himes et al., 1997).

Previously, prevalence studies were based on hemoglobin determinations and focused on the prevalence of anemia as an outcome of iron deficiency. However, the classic hematologic parameters of iron status (hemoglobin concentration, MCV, MCHC, transferrin saturation) show wide variation in normal subjects and marked overlap in iron deficient subjects (Hallberg et al., 1993). The use of these methods is valid in populations with severe and advanced iron deficiency, such as in developing countries.

In highly industrialized countries, such as Canada and the United States, the severity of iron deficiency is usually mild and the above methods lack sensitivity for use in epidemiological studies. Thus, an underestimation of actual prevalence may result. It is appropriate to include the measurement of serum ferritin to achieve a more valid assessment of the prevalence of iron deficiency. The relationship between serum ferritin and iron stores is well established; low serum ferritin values are seen only in iron deficiency (Walters et al., 1973). Also, each of the indices of iron status selected for the present study reflects a change in different iron compartments in the body and each is affected at different stages of iron depletion. Used singly, individual tests are not specific for iron deficiency as they are influenced by the coexistence of confounding factors (Preziosi et al., 1994). However, when used in combination their specificity is much higher (Yip & Dallman, 1996). Using a combination of measures not only provides a more accurate measure of iron status, but also allows for the determination of the stage of iron deficiency (Gibson, 1990a; Cook et al., 1987; Expert Scientific Working Group, 1985; Cook & Finch, 1979).

4. Iron Requirements

In normal healthy individuals, the highest demand for iron occurs during periods of rapid growth, such as infancy, childhood and adolescence. The increased need for iron is primarily due to the hemoglobin requirements of the expanding blood volume. Total daily iron loss in men amounts to ~1.0mg, while menstruating women require ~1.3mg/day to compensate for the iron lost in menstrual blood in addition to the normal daily losses (Yip & Dallman, 1996). Since males typically absorb ~6% and premenopausal females absorb ~13% of total dietary iron, the amount of iron needed daily is considerably higher than physiological requirements in order to maintain healthy iron nutriture (Yip & Dallman, 1996). The special concerns regarding the iron requirements of adolescents will be addressed below.

4.1 Adolescent iron requirements

Teenage girls require very high amounts of iron, particularly during stages of rapid growth. From the beginning of the growth spurt and for approximately two years beyond, gains in height and weight require additional iron for new tissue development. Throughout this period of growth and tissue accretion, an additional 0.35mg of iron in excess of normal physiological requirements is needed daily (Hallberg, 1992). The average annual weight gain of teenage girls between the ages of 14 and 16 is 3.8kg and they gain an average of 9kg during the year of peak growth (Hallberg, 1992). Consequently, adolescent females are susceptible to the development of a compromised iron status if the iron requirements for growth are not met. The estimated median daily requirement for iron calculated by Hallberg (1992) using data from the FAO/WHO Report (1988) is 1.73mg/day, considering the additional requirements related to growth. Menarche, which usually follows the growth spurt, places the adolescent female at an even greater risk for iron deficiency. If teenage girls cannot meet the increased demands for iron prior to menarche and already have a poor iron status, it may be difficult to establish and maintain a favorable iron balance once the additional demands associated with regular menstruation begin.

Adolescent males gain an average of 5.5kg per year between the ages of 12 and 16. During their peak year of growth, the average weight gain is 10kg (Hallberg, 1992). Hemoglobin concentration increases concomitant with sexual maturation and the expanding blood volume, resulting in a median daily requirement of 1.5mg/day or even higher (Brabin & Brabin, 1992).

The Canadian Recommended Nutrient Intake (RNI) for iron is based on a mixed diet that contains meat, poultry and fish as well as foods high in ascorbic acid (i.e. a diet of high iron bioavailability) (Health and Welfare Canada, 1990). The RNI for iron for adolescent females between the ages of 16 and 18 is 12mg/day (13mg/day between the ages of 13 and 15). Adolescent males between the ages of 13 and 18 have a RNI of 10mg/day (Health and Welfare Canada, 1990).

5. Iron Depletion

Iron status can be viewed as a continuum ranging from iron overload to degrees of iron depletion from various tissues. Iron balance implies an adequate amount of iron is being taken in to meet physiological demands. In a situation of prolonged negative iron balance, iron stores will eventually become depleted and the supply of iron to different tissues, including the erythron, will be insufficient. Although there is no evidence that an absence of iron stores is directly associated with negative consequences, it does indicate an undesirable and compromised supply of iron, which may have a negative impact on health (Bothwell, 1995; Hallberg, 1992). When iron stores become depleted, any further reduction in body iron is associated with a decrease in functional iron structures.

In healthy individuals with normal iron status, there is sufficient iron in the storage compartment to meet all tissue requirements and hemoglobin concentration is optimal. During negative iron balance, iron stores gradually decline. When iron stores are exhausted, an early stage of iron deficiency has been reached. The mildest state of iron deficiency is often referred to as *storage iron depletion* and is detectable when serum ferritin has dropped below normal (Bothwell et al., 1979a). Iron depletion causes no physiological impairments as the functional iron may not be affected (Herbert, 1992). However, iron intake and absorption must be sufficient to meet the physiological requirements of growth and to compensate for iron losses. A continued negative iron balance is detected by changes in other laboratory measurements, including transferrin saturation. This confirms the state of iron deficient erythropoesis, or *iron deficiency,* and indicates that the functional iron pool has been affected (Expert Scientific Working Group, 1985). In the state of iron deficiency, the amount of iron absorbed is insufficient to replace losses or meet the demands for growth and function. At this point, the storage iron compartment has been exhausted and iron supply to tissues is inadequate. Subsequently, the tissues and systems that require iron become functionally altered. The states of iron depletion and iron deficiency leave the affected individual with no iron reserves and subsequently, ill-prepared to overcome a physiological challenge to their iron stores.

Prolonged negative iron balance leads to the most severe form of iron deficiency, *iron deficiency anemia*. Hemoglobin begins to decline early in iron deficiency, however, the decline is not usually detected until hemoglobin concentration falls below established standards for a population (Hallberg, 1992). At this stage, an individual is classified as having iron deficiency anemia. The production of iron-containing functional compounds is impaired and red blood cells are hypochromic and microcytic (Herbert, 1992)

6. Populations at Risk of Compromised Iron Status

Several factors contribute to the development of iron deficiency and iron deficiency anemia (Table 1).

Table 1: Etiology of iron deficiency

ELEVATED IRON REQUIREMENTS	INSUFFICIENT IRON ABSORPTION
Growth Blood loss • Menstruation • Gastrointestinal tract (food sensitivity, hookworms, elite athletes) • Genitourinary tract • Respiratory tract • Blood donation Pregnancy	Diet low in bioavailable iron Impaired iron absorption • Intestinal malabsorption • Gastric surgery • Hypochlorhydria

Adapted from Centers for Disease Control and Prevention, 1998

Many populations are vulnerable to developing iron deficiency. Children under two years of age are at extremely high risk for iron deficiency because they are undergoing rapid growth and often have an inadequate intake of dietary iron (Centers for Disease Control and Prevention, 1998). Infants fed non-iron fortified formula or cow's milk are at a further increased risk as iron stores of full-term infants are exhausted by six months of age. Furthermore, early introduction of cow's milk may cause occult gastrointestinal bleeding (Yip & Dallman, 1996). Infants and preschool children may suffer developmental delays and impairments of height and weight gain and behavioural disturbances as a result of iron deficiency anemia. In this state of compromised iron status, children are also susceptible to lead poisoning since the gastrointestinal tract's ability to absorb heavy metals is increased (Goyer, 1995).

Adolescents comprise another group that is at high risk, as the demands of rapid growth can exceed the amount of iron obtained from the diet. In both sexes, the demand for iron is high in teenagers to meet the demands of increased blood volume and hemoglobin concentration. In males, the increment in lean body mass induced by the pubertal growth spurt imposes a drain on iron stores. Once beyond the peak growth period, the risk for iron deficiency subsides among boys. Recovery from iron deficiency that may have developed during the growth spurt is possible. Adolescent girls, however, usually reach menarche immediately after their peak pubertal growth (Gong & Spear, 1988) and the risk for becoming iron-compromised is further compounded. The increased stress on iron status imposed by regular menstruation continues throughout the adult woman's life until menopause. Vulnerable adolescent females with low iron reserves have a high likelihood of developing anemia should pregnancy occur. An additional risk factor for women of childbearing age is the propensity for heavy menstrual blood loss, which affects an estimated 10% of women (Bothwell et al., 1979b).

The most recent national estimate of iron deficiency prevalence in the United States is between 9% and 11% for girls between the ages of 12 and 19 (Looker et al., 1997). The prevalence of iron deficiency anemia in the same population is estimated between 2% and 3% (Looker et al., 1997). The prevalence of iron deficiency and iron deficiency anemia among males of the same age is <1% (Looker et al., 1997). Similar data are not available for Canadian adolescents.

Elite athletes, especially long-distance runners and competitive swimmers, have a propensity to develop iron deficiency, believed to be due to gastrointestinal blood loss (Eichner, 1992; Weaver & Rajaram, 1992; Stewart et al., 1984). Multiparous women, as well as women with low iron intake and a previous diagnosis of anemia are also at elevated risk for iron deficiency. Oral contraceptive use is associated with decreased risk for iron deficiency as menstrual blood loss is decreased by half (Yip & Dallman, 1996).

Pregnancy imposes additional demands for iron because of the expanding blood volume, growing fetus, placenta and other maternal tissues. Despite the cessation of menstruation and the increased mucosal absorption of iron throughout pregnancy, many women cannot achieve adequate amounts of iron from diet alone, especially during the last half of pregnancy. Women in a lower income bracket are particularly vulnerable to developing iron deficiency anemia during pregnancy (Perry et al., 1995).

In healthy adult men and post-menopausal women, iron deficiency anemia is uncommon as the storage compartment of iron will continue to expand throughout adulthood. The risks for compromised iron status in this population are not usually due to inadequate dietary iron. More common causes of iron depletion and iron deficiency anemia are gastrointestinal blood loss, chronic disease and inflammatory conditions (Centers for Disease Control and Prevention, 1998; Yip & Dallman, 1996).

7. Manifestations of Iron Deficiency

Although more distinguishable in infancy and early childhood, the manifestations of iron deficiency in other age groups are often subtle and non-specific. Anemia is the most widely publicized manifestation of iron deficiency. In its early stages, iron deficiency anemia is of little consequence since compensatory mechanisms will ensure oxygen delivery to tissues. In more advanced stages of anemia, oxygen delivery is greatly reduced and more overt functional impairments ensue (Finch & Cook, 1984). Iron deficiency can impair mental functions such as learning and the ability to concentrate, physical and work performance, and it can decrease exercise performance, especially endurance exercise (Yip & Dallman, 1996; Scrimshaw, 1984). Maintenance of body temperature can also become impaired, as well as immune function and resistance to infection (Cook & Lynch, 1986). Other symptoms of anemic individuals include general weakness and fatigue, pallor, dyspnea, rapid or irregular heart beat, loss of appetite, vague gastrointestinal symptoms, and dull, brittle fingernails (Fairbanks, 1994).

8. Treatment of Iron Deficiency

Iron deficiency can be prevented by consuming adequate quantities of foods with high iron bioavailability. Iron absorption can be improved by consuming foods that enhance iron absorption and avoiding iron inhibitors at meals. The treatment of iron deficiency anemia requires iron supplementation, as the amount of iron obtained from the diet is usually insufficient to correct this condition (Fairbanks, 1994). Oral iron therapy is the most common method of supplementation and the dose of iron depends on the expected hematologic response, the amount of iron required to achieve this effect and the rate of iron absorption from the iron supplement selected (Fairbanks, 1994). Iron from tablets or liquid supplements is absorbed twice as well when taken between meals rather than with meals, and iron absorption is enhanced when taken with ascorbic acid (Yip &

Dallman, 1996; Fairbanks, 1994). In addition to treating the deficiency, iron therapy should also treat the cause.

B. FACTORS INFLUENCING THE NUTRITIONAL STATUS OF ADOLESCENTS

Several physiological, psychological and sociocultural factors contribute to behaviours that ultimately influence the nutritional status of adolescents. The teen years signify a period of physical and emotional maturation, with many issues surrounding the rapid development that adolescents undergo. Table 2 highlights some common concerns encountered during adolescence that have the potential to influence dietary intake and nutritional status.

Table 2: Factors affecting adolescent dietary intake and nutrition status

PHYSIOLOGICAL CHANGES	NUTRITIONAL CONCERNS	SOCIOCULTURAL INFLUENCES
◆ Onset of puberty • Growth spurt • Expanding cell mass • Weight increase • Height increase • Bone accretion • Altered body shape • Hormone production reproductive organs skin changes brain function ◆ FEMALES • Fat accumulation • Menarche ◆ MALES • Accretion of lean body mass	◆ Energy intake ◆ Protein intake ◆ Appropriate balance & variety ◆ Dietary deficiencies • Iron • Calcium • Zinc • Vitamin A • Folate • B vitamins • Fibre	◆ Elevated independence ◆ Social & cultural norms and values ◆ Personal experience ◆ Peer influence ◆ Media influence • Food choices • Body perception ◆ Personal & sociocultural expectations of appearance ◆ Lifestyle ◆ Family influence & personal knowledge ◆ Ethnic, religious & personal beliefs ◆ Food availability

Many of these issues are intimately connected and have a direct bearing on the eating behaviours and nutritional status of teenagers. The following section describes the influences of growth, body image, behaviours, lifestyle and specific nutrients of concern, as well as the influences of health/medical factors.

1. Adolescent Growth and Development

Adolescence is a time of rapid physical growth. The growth spurt, or period of maximal rate of growth, occurs in American boys at a mean age of 13.5 years and in girls at a mean age of 12.5 years, and is approximately two years in duration (Underwood, 1991). Teenagers attain approximately 15% of their maximal height and 45% of their final adult skeletal mass between the ages of 10 and 15 (Gong & Spear, 1988). During the growth spurt, the accretion of nitrogen and minerals, such as calcium and iron, are two to three times greater than normal to allow for the increment in lean body weight (Forbes, 1991). Lean body mass is achieved by approximately age 18 in girls and age 21 in boys (Forbes, 1991). This underscores the elevated need for nutrients and minerals, not only during the growth spurt in early adolescence, but also throughout the entire second decade of life. In terms of body composition, males experience a more intense period of growth and gain more lean body mass, whereas girls gain more body fat (Forbes, 1991). In addition to a proportionately higher muscle mass, males also develop greater red blood cell mass compared to girls (Gong & Spear, 1988). This difference is largely due to differences in hormone levels and activity.

Normally, the onset of puberty occurs in early adolescence and is dependent on growth hormone, insulin-like growth factor and nutrients. Variations in nutritional status as well as energy and protein in the diet are as influential as growth hormone in modulating the different growth factors (Underwood, 1991). The onset of menstruation usually occurs at age 13, approximately one year after peak height velocity (Gong & Spear, 1988). Among both genders, this period of rapid growth and physical and physiological change has many implications in terms of food choices and nutritional status.

2. Body Shape and Eating Concerns Among Adolescents

Teenagers are undergoing many psychological changes, establishing ideals and norms and achieving heightened self-awareness and self-concept. In the search for their identity and independence, and concern for their appearance and peer acceptance, nutrient intakes and food choices are inevitably influenced. Despite the strong genetic component in rate of growth, height, weight, body shape and breast size, it is difficult for many adolescents to accept the changes of puberty and the growth spurt. Drastic nutritional measures are often taken to try to mould the body into a cultural ideal.

2.1 Peer influence

Peers are highly influential with regard to food choices. As a consequence of peer influence, unhealthy eating may become regarded as socially acceptable, as indicated in a recent survey conducted in the United States (Evans et al., 1995). In this survey, Evans and colleagues (1995) assessed over 5000 adolescents' perceptions of how their peers viewed various health-related behaviours. Of the 11 behaviours evaluated, "eating healthy food" was the least valued and "female peers controlling their weight" was the most valued behaviour for peers to participate in. The teenagers also indicated attractiveness was a more powerful motivator for behaviour change than "healthfulness" (Evans et al., 1995).

2.2 Influence of the media

Mass media and entertainment influence food choices as well as self-image. The media is also highly influential in perpetuating societal preferences for both female and male body shapes. Recent research by Field and colleagues (1999a) suggests there is a direct relationship between print media and weight and body shape perceptions of young girls. Discontent with body weight and shape was strongly related to the frequency of reading fashion magazines. A positive association exists between frequency of reading fashion magazines and the prevalence of dieting for weight loss because of a magazine article. There is also a positive association between reading these magazines and wanting to lose weight because of photos depicting the ideal body. Participants revealed that their idea of the perfect body shape is influenced by magazine pictures (Field et al., 1999a).

Several other recent research studies have established a link between the mass media and models who represent a thin ideal, sociocultural expectations, and unhealthy body attitudes among young women (Pinhas et al., 1999; Wertheim et al., 1997; Stice et al., 1994). The goal of attaining an ideal body may dominate one's food choices.

2.3 Adolescent self-concept

It has been extensively documented that young females are preoccupied with their weight status. Many studies have demonstrated female body dissatisfaction and the subsequent diet restriction, unhealthy eating practices and alteration of food perception (Story et al., 1998; French et al., 1994; Serdula et al., 1993; Felts et al., 1992; Story et al., 1991; Casper & Offer, 1990; Moore, 1990; Richards et al., 1990; Davies & Furnham, 1986). A study of adolescent girls conducted by Moses and colleagues (1989) revealed that half of the girls who were identified as underweight were "extremely fearful" of becoming overweight and had a distorted perception of ideal body weight.

Among boys, weight dissatisfaction is prevalent, although to a lesser extent than their female peers. Some research indicates a small proportion of adolescent males perceive themselves as being too fat and practice weight control measures (Story, 1998; Felts et al., 1992). However, other studies indicate males tend to perceive themselves as normal weight or underweight and possess the desire to gain weight (Serdula et al., 1993; Moore, 1990; Miller et al., 1980; Huenemann et al., 1966). A recent Canadian survey indicated 31% of adolescent males were trying to gain weight, showing an increasing trend throughout high school from 18% in grade seven to 41% in grade 12 (The McCreary Centre Society Adolescent Health Survey, 1993). Overall, weight and body image concerns and the related dieting behaviours are far less prevalent in adolescent boys compared to girls (Strauss 1999; Story et al., 1998; French et al., 1994; Serdula et al., 1993; Felts et al., 1992; Story et al., 1991; Casper & Offer, 1990; Moore, 1990; Richards et al., 1990).

It is apparent from the existing body of research that many health and eating behaviours are appearance-driven. Subsequently, many teenagers engage in practices to control food intake and body weight, including dieting, eating less meat, adopting vegetarianism, skipping meals, and eliminating entire food groups from their diets

(French et al., 1995; French et al., 1994; Felts et al., 1992). The focus on appearance and drive for thinness seems to represent a cohort perspective. Adolescents value appearance and find it difficult to differentiate between healthy weight standards and the unrealistic ideals often held by this age group (Felts et al., 1992). Many adolescents, especially females, engage in unhealthy eating practices, thereby exposing themselves to health risks which can be detrimental to nutritional status, and more specifically, to iron status.

3. Behavioural and Lifestyle Influences on Adolescent Nutritional Status

The eating habits of adolescents have undergone major changes over the past 50 years. These changes are a result of three principal influences: increased independence and freedom to make personal decisions, greater purchasing power to acquire meals, snacks and beverages, and access to and the influence of media (Anderson, 1991). Teenagers experience an increasing drive for independence, often with support from parents. Throughout the transition from childhood to adulthood, food may be one of the few common threads remaining between parents and teenagers. However, as adolescents develop intellectually they may form different opinions which can result in meal time struggles and conflict. Several factors outside the family can have an impact on dietary intakes. These include peer influence, unpredictable schedules and eating style.

3.1 Peer influence

Adolescence is a time of intellectual growth and maturity. Often for the first time, teenagers are afforded the freedom to make choices in all aspects of their lives, including those related to food. Teenagers are primarily responsible for their own food intakes, which may be influenced by food availability and convenience, time availability, food preferences, income, peer influence, and the influence of media and advertising. Often the influence of peers exceeds the influence of parents at this age (Frank, 1997). Many adolescents eat meals away from home and invariably develop irregular eating habits due to part time jobs, social activities and involvement in sports and other extracurricular activities. Although the influence of sports involvement and part-time jobs was not correlated with dietary quality in adolescent girls in Ontario (Absolon et al., 1988), other research indicates the general fast pace of adolescent life does contribute to poor diet quality (Story & Resnick, 1986).

3.2 Busy schedules

Story and Resnick (1986) assessed the nutritional views and opinions of students in several high schools throughout Minnesota. Small group discussions revealed that teenagers were well informed about good health and nutrition practices, however their eating habits were generally poor. The participants themselves indicated their poor eating habits included skipping meals, snacking too much and eating unbalanced meals. The reasons cited for poor nutritional choices included the lack of time to eat properly or plan nutritious meals, the lack of convenience of eating properly, and the lack of a sense of urgency to adopt more healthy eating habits.

The generally faster pace of life for the entire family may also lead to sporadic eating habits and poor quality diets in teenagers. There has been a shift in the traditional family structure over the past few decades, with more single parent households and more women working outside the home. Complicated family schedules as well as busy individual schedules can interfere with family meals and ultimately can decrease diet quality (Story & Resnick, 1986). Despite their perception that fast foods are less healthy, teenagers turn to "junk food" primarily because of taste, but also because they are convenient and a necessary part of their busy lifestyles (Story & Resnick, 1986).

3.3 Vegetarianism

There has been a recent increase in the popularity of vegetarianism, especially among females. Research in Britain indicates a threefold rise in the proportion of women aged 16 to 64 years who avoided eating meat between 1988 and 1994 (British Nutrition Foundation, 1995). The latest research from Worsley and Skrzypiec (1998) found that teenage vegetarianism is primarily a female phenomenon. In their study of prevalence of vegetarianism in high school students in Southern Australia, meat exclusion from the diet was four to five times more common in girls compared to boys (Worsley & Skrzypiec, 1998). Neumark-Sztainer and colleagues (1997) found a similar high prevalence among teenage girls compared to boys in a North American population. 81% of the vegetarians surveyed were female (Neumark-Sztainer et al., 1997).

There are definite concerns about the adequacy of a meatless diet, especially among adolescent girls because of their high nutritional requirements. The bioavailability of a number of nutrients are altered by the components of a vegetarian diet, thereby potentially compromising mineral status (Freeland-Graves, 1988). Inadequate intakes of iron (less than 2/3 of the RNI) have been reported in adolescent female vegetarians and semi-vegetarians (Donovan & Gibson, 1996). Another cause for concern is the low bioavailablity of nonheme iron. In addition, plant based sources of iron are further hindered due to the presence of iron-absorption inhibitors such as fibre, phytates and polyphenols (Bothwell, 1995).

Several reasons exist for adopting a vegetarian way of eating including moral, ethical, symbolic and health reasons. Among teenagers, the vegetarian choice may be related to identity negotiation and the expression of radical values. It may be an opportunity to assert independence or for self-identification. It may be a way to express oneself through choosing eating patterns different from family or society. This expression may be particularly relevant for adolescent females who feel most of their options in life are determined by family and society (Worsley & Skrzypiec, 1998). It has also been postulated that teenagers choose vegetarianism as a means of rejecting parental values. More commonly, teenagers, and especially girls, adopt a vegetarian style diet for weight loss (Worsley & Skrzypiec, 1998; Neumark-Sztainer et al., 1997; Ryan, 1997; Worsley & Skrzypiec, 1997). Some individuals may use vegetarianism as a socially acceptable way to avoid fat and restrict energy intake (Neumark-Sztainer et al., 1997). Furthermore, a recent study conducted in Canada indicates that the adoption of a vegetarian eating style may be an attempt on behalf of some individuals, including males, to hide dieting behaviours from others (Martins et al., 1999). Given the association between meat avoidance and the desire to be thinner among adolescent females, it seems unlikely that they would take measures to compensate for the lack of heme iron, greatly increasing the risk for nutrient deficiencies.

Despite the many health benefits of vegetarianism (Thorogood, 1995), adolescents, and especially female adolescents, are at an increased risk for compromised iron status when restricting and eliminating meat. In vegetarian women, mean serum ferritin concentrations were significantly lower when compared to omnivorous controls (Ball & Bartlett, 1999). This finding would be exaggerated in adolescent females due to the higher iron demands of growth and the initiation of menstruation. Although there are several dietary strategies to improve iron absorption from a vegetarian diet (Gibson et al., 1997), it is not known if adolescents are aware of these or if they practice them on a regular basis. If not, they are more vulnerable to iron deficiency.

4. Nutrients of Concern Among Teenagers

The changes in body composition and rapid tissue synthesis during the teen years rely largely on energy, iron and protein. The increases in lean body mass, blood volume, red blood cell counts and hemoglobin levels increase iron requirements in teenage boys, and to a lesser extent in teenage girls. The onset of menarche and subsequent regular menstruation increase the iron requirement for adolescent females. In addition to iron, several other nutrients are required in larger amounts during adolescence to balance growth requirements. These include energy, protein, calcium, zinc, folate, vitamin A, B vitamins and fibre.

Generally, females tend to consume lower amounts of nutrients compared to males. According to recent North American data, average energy intake of females is 2/3 that of males of the same age (Norris et al., 1997; Shatenstein & Ghadirian, 1996; Salz, et al., 1983). Results of large North American epidemiological surveys indicate that teenagers, and especially teenage girls, may be getting inadequate amounts of iron, calcium and folate (Devaney et al., 1995; Alaimo et al., 1994; Chapman, 1994; Absolon et al., 1988; Seoane et al., 1985). The gender difference in dietary intakes can be explained by the estimated prevalence of dieting and different requirements for males and females. The Youth Risk Behavior Survey conducted in the United States (1993) indicates almost 2/3 of adolescent females were attempting to lose weight, compared to only one quarter of males in grades nine through 12 (Kann et al., 1995).

5. Other Influences on Iron Status in Adolescence

In addition to the factors outlined above, there are several other practices that influence iron status among adolescents. These include exercise, infection, smoking cigarettes and oral contraceptive use. Each of these factors are discussed in the following section.

Teenage athletes may be at increased risk for iron deficiency due to normal tissue demands, increased red blood cell formation and lean mass accretion combined with poor dietary habits and red blood cell destruction. A research study conducted by Lyle and colleagues (1992) indicates even moderate amounts of short term aerobic exercise can compromise iron status in adolescent females. A recent study conducted with older adolescent females demonstrated a decline in hemoglobin in females participating in a long-term moderate exercise program, although the results cannot be attributed solely to exercise effects (Rajaram et al., 1995). In both studies, iron status did not change in comparative groups receiving iron supplements. Iron supplementation appears to protect exercisers against the iron-compromising effects associated with training, especially when the iron is supplemented through heme sources (Rajaram et al., 1995; Lyle et al., 1992). Generally, teenage boys are less susceptible to the development of iron depletion with moderate exercise training, as they are less frequently iron deficient. However, vigorous weight training can result in decreased iron stores (Cook, 1994).

There are several potential causes for the iron deficiency that results from intense exercise and endurance training (such as elite runners). Iron may be lost through sweat, the gastrointestinal tract and hematuria. In addition, the increased demand for total body hemoglobin and poor dietary iron intakes can further elevate the risk of developing iron deficiency in athletes (Position of the American Dietetic Association, 1987).

Sports anemia is also a concern among adolescent athletes. This results from the acute stress response to vigorous exercise and the destruction of erythrocytes concomitant with the expansion of plasma volume (Raunikar & Sabio, 1992; Position of the American Dietetic Association, 1987). The subsequent decrease in hemoglobin produces normocytic, normochromic anemia which is associated with athletic training. Sports anemia is not an anemia of iron deficiency, although insufficient iron intakes may contribute to its development (Position of the American Dietetic Association, 1987).

It has recently been shown that mild infections, such as a sore throat with a slight fever can have a dramatic influence on serum ferritin, wherein ferritin is elevated with no actual change in the storage iron compartment (Hulten et al., 1998). Although the causative factors remain unclear, Hulten and colleagues speculate that ferritin is affected indirectly through the reticulo-endothelial system. Inflammation and infection elicit effects on the reticulo-endothelial system, which in turn influences the labile iron pool and thus impact the storage iron compartment, the source of ferritin. Reeves and colleagues (1984) speculate that mild infections predispose individuals to iron deficiency due to decreased iron absorption. However, infants were examined in this study and therefore, the conclusions are not necessarily pertinent to adolescents. Hulten's research team investigated this association in Swedish teenagers and discovered that recent mild infections elevated serum ferritin independent of iron status, thus underscoring the importance of taking medical history (including incidence of colds and infections) into consideration when assessing causes of iron deficiency in populations (Hulten et al., 1998).

Cigarette smoking has been shown to cause an upward shift in mean hemoglobin values, a relationship that is directly related to the number of cigarettes smoked per day (Nordenberg et al., 1990). The carbon monoxide from inhaled cigarette smoke binds to hemoglobin to form carboxyhemoglobin, an inactive form of hemoglobin with no oxygen-carrying capacity (Collier, 1976). In order to compensate for the overall reduction in oxygen delivery to tissues, higher hemoglobin levels are established and maintained in smokers (Nordenberg et al., 1990; Smith & Landaw, 1978). Based on data from NHANES II, using normal hemoglobin ranges to detect anemia when comparing smokers to non-smokers should be employed with caution. Alternately, cutoffs can be adjusted to compensate for the masking effect of smoking (Nordenberg et al., 1990).

Oral contraceptive use in female adolescents appears to offer some protective effects against iron deficiency not only from the reduction in blood loss, but also due to altered hormone status. Mooij and associates (1992) determined that endogenous and exogenous steroid hormones influence serum iron levels differently. Women who used oral contraceptives had higher serum ferritin, serum iron and TIBC when compared to non-oral contraceptive users (Mooij et al., 1992). This can be explained by the alteration of intravascular water and the subsequent compensatory increase in iron absorption among women who use oral contraceptives. Although oral contraceptive use appears to improve iron balance, the authors speculate that menstrual bleeding and dietary intake are more influential on iron status.

The number of years since menarche has been evaluated in relation to body iron stores. A research team in Japan conducted a longitudinal assessment of girls who were 10 years old at the beginning of the study. The participants were followed for eight years to determine the relationship between biochemical and hematologic measurements, menarche and stature. Kagagimori and colleagues (1988) found that adolescent girls had low serum ferritin three years after menarche, which is an early indicator of storage iron depletion. After the third year post-menarche, serum ferritin began to rise (Kagagimori et al., 1988). This study revealed that storage iron decreased significantly due to the occurrence of menarche rather than the effects of the increase in height velocity associated with the growth spurt.

Several factors play a role in the food choices, and subsequently influence the nutritional status, of adolescents. The period of rapid growth and development that occurs in the teenage years imposes physiological demands and increases nutrient requirements. External influences, such as those of peers, the media and busy lifestyles affect dietary choices. Internal influences such as personal beliefs and values and self-concept also affect food selection. In addition, chosen behaviors including exercise and cigarette smoking also have an impact on nutritional status, and more specifically, iron status. Many of these factors function synergistically to influence the nutrient intake and overall health of adolescents.

C. ASSESSMENT OF DIETARY INTAKE, LIFESTYLE BEHAVIOURS AND MEDICAL FACTORS IN RELATION TO IRON STATUS

Various factors affect the food choices, nutrient utilization and nutritional status of teenagers. Several techniques can be employed to determine nutrient intake. Medical history and current health as well as lifestyle behaviours can also have a profound influence on iron nutriture. A multi-factorial approach should be utilized to account for the complex interaction these factors play in the iron status of adolescents.

1. Dietary Intake

Accurate assessment of the dietary intake of adolescents can be problematic due to the diverse nature of their eating habits. Within any given day in a teenager's life, eating may take the form of several quick snacks or a few solid meals. Eating may be a group decision or an independent endeavor, a positive or negative experience. There may be high day-to-day variability and high variability between individuals. A variety of dietary assessment techniques are available to quantify nutrient intake for the study of adolescent nutritional status.

Adequacy of nutrient intakes is often determined by establishing a cutoff value below the RNI (Gibson, 1990b). This technique is appropriate considering the RNI is established at a level high enough to meet the requirements of almost all individuals within specified groups (Gibson, 1990b). Although there are no clearly defined rationale for selecting the cutoff value, 2/3 of the recommended nutrient intake has been used previously in similar studies (Siega-Riz, et al., 1998; Zive et al., 1996; Anderson et al., 1982). Using a cutoff value below the RNI reduces the possibility of overestimating the actual prevalence of intakes below individual requirements (Gibson, 1990b).

1.1 Measuring dietary intake of individuals

Research goals must be carefully considered when selecting the most appropriate dietary assessment technique, taking into consideration the desirable and undesirable aspects of each method (Wolper et al., 1995). The technique selected to assess nutrient intake should not interfere with normal dietary habits and thus not influence the parameter of interest (Westerterp, 1994). Also, the data collected should be representative of usual or habitual intake (Torun et al., 1996).

The standard methods for measuring food consumption can be categorized into two groups: (1) quantitative daily consumption methods (one day recalls or records) and (2) retrospective methods (diet history and food frequency questionnaire (FFQ)) (Gibson, 1990c). Quantitative methods measure the quantity of foods consumed over a defined period of time. Estimates of actual or usual intakes can be obtained from this type of data. The evaluation of usual intake is particularly important when analyzing relationships between habitual diet and biological parameters (Gibson, 1990c). Retrospective methods gather diet information on the patterns of food consumption over a longer, less precisely defined period of time. These methods are most frequently used to assess usual intake of foods or specific groups of foods. With modification, however, retrospective methods can provide information on usual nutrient intakes (Gibson, 1990c).

1.2 Food frequency questionnaire

A FFQ is designed to collect qualitative, descriptive information about usual food consumption patterns over an extended period of time (Gibson, 1990c; Block & Hartman, 1989). The questionnaire consists of a list of foods and a set of frequency-of-response categories. The list of foods may be extensive or may focus on specific groups of foods, particular foods or foods consumed occasionally in association with special events or seasons (Anderson, 1986). Comprehensive FFQs typically should contain 100 or more food items to capture the range of foods contributing to the variety of different nutrients in the diet (Thompson & Byers, 1994).

The aim of the FFQ is to determine the frequency of consumption of certain foods or food groups during a specified time span (previous month, six months, year) (Gibson, 1990c). Semiquantitative food frequency data can be obtained when the FFQ is modified to attempt to quantify usual portion sizes of foods. In this case, the participant has the opportunity to indicate both the frequency and quantity (measure, size, number of portions) of foods usually consumed in a specified time period (Gibson, 1990c). Although the semiquantitative FFQ collects more information regarding food portions, it allows only limited quantification of serving sizes (Thompson & Byers, 1994).

This dietary assessment tool is inexpensive to administer, can be completed in a relatively short period of time and is easy to standardize. The data for the FFQ can be obtained through a standardized interview or self-administered questionnaire, both taking approximately 15 to 30 minutes to complete, depending on the number of items in the food list. The FFQ has a low response burden compared to most other dietary assessment methods. It is frequently used in epidemiological research to determine if associations exist between dietary habits and disease (Hirayama, 1981). The self-administered FFQ is generally the only method that is logistically feasible for large studies (Jain et al., 1996). This measurement technique provides reliable information on specified food items from the remote past, as well as the recent past (Hislop, 1990). Generally, the FFQ is the most convenient and cost-effective method of assessment of usual dietary intakes in large groups.

1.3 Measuring dietary intake with the food frequency questionnaire

The FFQ typically utilizes precoded forms that incorporate a selected list of foods arranged in food groups of comparable nutrient content (Wolper et al., 1995). The questions address portion sizes and frequency of consumption for each food item; specific amounts are usually described in common household measurements. Individuals are asked to indicate how often they eat each food and choose the time period (per day, week, or month). Data from the questionnaire can be used to rank participants into broad categories of low, medium and high intakes of certain foods (Gibson, 1990c). Alternatively, the data can be scored based on frequency of consumption of certain food groups (Gibson, 1990c). These scores can then be examined in relation to personal demographics (age, weight, marital status), psychosocial influences (level of education, income), geographic distribution and season. When usual intake is quantified using the FFQ, nutrient intakes can be calculated from the data. Nutrient scores for each person can be calculated by multiplying the frequency of consumption of each food item by the nutrient content of the average portion specified (Gibson, 1990c). The nutrient content of foods is obtained from appropriate food composition data (Russel-Briefel et al., 1985).

1.4 Variation in the food frequency questionnaire

The precision (reproducibility/reliability) of dietary assessment methods is difficult to determine since it is almost impossible to obtain the same results on repeated administration due to normal day to day variety in food selection. The reproducibility of a questionnaire does not indicate whether the instrument is producing the correct answer, only whether it is producing the same answer (Block & Hartman, 1989). Precision is influenced by measurement error and the true variation in daily food consumption (Hankin et al., 1967). Studies assessing the precision of the FFQ are limited. A study conducted by Acheson and Doll (1964) indicated that after three months, 90% of the responses on a repeated FFQ did not differ from its original administration. Willett and Stampfer (1986) reported that after one year, re-administration of a self-administered semiquantitative FFQ resulted in similar mean daily nutrient intakes, with high correlation ($r = 0.55$ to 0.74) for several nutrients. A reproducibility study conducted by Mares-Perlman and colleagues (1993) indicated good agreement between two food frequency questionnaires administered in the same population at a three-month interval. If measurement errors are minimized, the precision of the assessment of nutrient intakes is a function of the overall true variability in nutrient intakes (Sempos et al., 1985). The FFQ generally demonstrates good precision when appropriate instruction is provided prior to its administration.

1.5 Validity of the food frequency questionnaire

Validity describes the extent to which a dietary assessment method measures what it is intended to measure (Block, 1982). The absolute truth about usual dietary intakes is never known, therefore these types of dietary methods are the most difficult to validate. Since the absolute validity of dietary data is difficult, if not impossible to determine, researchers instead measure "relative validity". This approach allows the researcher to test the instrument of interest against another reference method which has a greater degree of demonstrated validity (i.e. diet records) (Block & Hartman, 1989).

Mullen and colleagues (1984) tested the validity of the FFQ against actual food intakes at each meal for 28 consecutive days. The results suggest most individuals can accurately estimate food intake using a FFQ. In another study, the FFQ was the only method that correlated significantly with total energy expenditure when compared to weighed seven day food intake assessment and 24 hour recalls in women (Sawaya et al., 1996). Two separate studies demonstrated the FFQ had higher correlations for several nutrients compared to an interviewer administered diet history, when using food records as the "gold standard" of comparison (Jain et al., 1996; Block et al., 1992).

Among adolescents, several studies have utilized FFQs to assess how often they eat specific foods. The time reference has been identified as a factor that may influence dietary intake responses in the adolescent population (Frank, 1997). However, data from Australia, Europe and the United States indicate teenagers can accurately describe the foods they eat using recollection-based techniques when appropriate prompts and adequate instruction are provided (Andersen et al., 1995; Karvetti & Knuts, 1992; Jenner et al., 1989; Baronowski et al., 1986; Persson & Calgren, 1984). In addition, Rockett and colleagues (1997) conclude that a simple self-administered FFQ can accurately assess adolescents' diets over the past 12 months.

Other potential errors associated with the FFQ include questionnaire design and quantification factors, inter- and intra-variability of intakes, gender and age of participant, day-of-the-week effects, seasonal effects, and training effects (Gibson, 1990c; Block & Hartman 1989). These errors can be minimized if they are taken into consideration in the initial design stage of the dietary assessment protocol, and if the protocol is maintained throughout the study period.

Several measurement errors are characteristic of all dietary assessment methods and include respondent bias, interviewer bias, respondent memory lapse, incorrect estimation, omission of supplement usage, deliberate omission of food items, coding errors, computation errors, and the "flat slope syndrome" (low intakes are overestimated and high intakes are underestimated) (Livingstone, 1995; Gibson, 1990c). Sources of error in determining nutrient content of food from food composition data banks include inadequate sampling protocols, inappropriate analytical methods, lack of standardized conversion factors, inconsistencies in terminology, incorrect description of foods, and inconsistencies in genetic, environmental, food preparation and processing factors (Gibson, 1990c).

There is no superlative method for assessing food or nutrient intakes. The objectives of the research study are of primary importance in determining the most appropriate assessment tool (Gibson, 1990c). All methods currently available are subject to systematic errors, and none can prevent participants from altering their usual food choices. Ultimately, results are dependent upon the motivation, compliance and ability of participants to accurately report habitual food intake (Torun et al., 1996). Based on its demonstrated precision and relative validity, time efficiency and cost effectiveness, the FFQ is the best assessment technique for assessing usual dietary intakes of large populations.

2. Validation of the Food Frequency Questionnaire

Considering the potential problems related to semiquantitative FFQs, findings based on the data collected should be viewed with caution. Data collected with FFQs can demonstrate wide variations in nutrient intakes. Although the reasons for this phenomenon are not clear, dietary standardization substudies (calibration studies) within the context of larger studies can elucidate some of the sources of variance (Kushi, 1994). Liu (1994) concludes that whenever possible, a calibration study should be conducted to evaluate the semiquantitative food frequency method within the population of interest in order to determine its relative validity. The purpose of these studies is to obtain an estimate of the magnitude of measurement error through providing a quantitative assessment of food intake in a sample of the study population (Kushi, 1994). The substudy should provide a reasonable quantitative representation of the diet intake patterns for the larger cohort (Kushi, 1994). The results of any diet assessment study are never truly valid, however standardization studies provide an estimate of the ability of other dietary assessment techniques to estimate the same underlying truth.

Food recording methods are often used in calibration studies to assess the relative validity of FFQs (Pomerleau et al., 1998; Bright-See et al., 1994; Thompson & Byers, 1994; Mares-Perlman et al., 1993; Russell-Briefel et al., 1985). The diet record is unlikely to overestimate the validity of the test instrument because it relies on recording a pattern of dietary intake. Thus, it is thought to provide the best approximation of a person's "true" usual nutritional intake and is considered a superior technique for this purpose compared to other methods, provided the diet record is administered meticulously (Potosky et al., 1990; Block & Hartman, 1989).

The diet record technique provides a measure of current food intake, and requires that all foods consumed are recorded at the time of consumption for a specified time frame (usually three, four or seven days) (Livingstone, 1995). Estimated food records are one technique for recording food intake. Detailed descriptions of all foods and beverages (including brand names) and their method of preparation are recorded. Composite dishes require that the participant record the recipe, the number of portions it yields and the amount consumed (Gibson, 1990c). Food portion sizes are estimated using household measuring cups and spoons when possible. Otherwise, portions are estimated based on the "best guess" of the trained participant. Appropriate detailed instruction is essential for the success of this technique. Portion size measures are then converted into weights and volumes by the investigator before calculating nutrient intakes (Gibson, 1990c).

The number of measurement days in an estimated food record varies. Seven measurement days are considered optimal for proper representation of actual intakes. However, the number of measurement days required to estimate usual intake varies by nutrient (Basiotis et al., 1987). Also, respondent burden and subsequent compliance must be taken into consideration (Livingstone, 1995). A study conducted by Gersovitz and associates (1978) suggests the records from the first two days were more valid than from the last three days when compared to known actual intakes in a validation study of seven day estimated food records. Consequently, shorter assessment periods may be selected ranging from two to five days (Gibson, 1990c). Weekend days should be proportionately included to account for day-of-the-week effects on food intakes (Gibson, 1990c). Generally, food records assess usual intakes of individuals and demonstrate good precision and validity (Gibson, 1990c).

3. Lifestyle Behaviours and Medical Factors

There are many potential influences on iron status, including specific lifestyle behaviours and medical factors. The factors addressed in the present research project include dieting, vegetarianism, blood donation, exercise, vitamin/mineral supplement use, cigarette smoking, recent infection, previous diagnosis of anemia, and heavy menstrual flow and number of years since menarche in females. In order to account for these, information should be collected and analyzed in relation to iron nutriture.

3.1 Techniques for measuring lifestyle behaviours and medical factors

In order to obtain useful data and maximize the effectiveness of a questionnaire, it is first necessary to identify the type of information that would be most useful and address specific questions of interest. Secondly, the wording for questions should be carefully selected such that they will be clear and easily understood by participants and obtain the information desired.

The general procedure for questionnaire development of this nature involves three defined stages. Stage one involves the assembly and utilization of a study group for initial determination of the type of information desired to address the relevant issues as they relate to iron status. The second stage involves the formation and utilization of focus groups to construct survey items that address identified areas of interest. The participants selected should be similar to the target population and can assist in writing questionnaire items that can be answered by participants in the larger study. Focus groups are valuable in identifying inappropriate items and can help clarify the meaning of questions as well as scrutinize the survey for content validity. The third stage involves pilot testing the questionnaire. This is an important step to assess problems in questionnaire administration and to standardize the administration protocol (Perry et al., 1985).

D. SUMMARY OF LITERATURE

The physiology of iron metabolism and absorption is a complex and multi-faceted process influenced by many dietary and non-dietary factors. Iron status is also subject to many diverse influences, and impaired iron status has far-reaching and potentially serious health consequences. Clearly, adolescents, and particularly female adolescents, possess many unique physiological and social challenges, which undoubtedly influence iron nutriture. The paucity of existing research on the nutritional status, and more specifically the iron status, of adolescents in Canada merits further investigation. This study assessed the iron intakes and iron status of adolescents and evaluated potentially influential lifestyle behaviours and medical factors.

CHAPTER THREE
EXPERIMENTAL DESIGN AND METHODOLOGY

A. ETHICAL APPROVAL

The present research study received ethical approval from the University of Alberta, Faculty of Agriculture, Forestry and Home Economics Human Ethics Review Committee. The Certificate of Approval from the University of Alberta is provided in Appendix A. Approval to conduct this research in local schools was granted by the Associate Dean of Research, Cooperative Activities Program (CAP), University of Alberta (Appendix B). Approval to conduct this research in schools under the jurisdiction of the Edmonton Public Schools was provided by the Research Liason, Edmonton Public Schools, Edmonton, Alberta (Appendix C). Written, informed consent was obtained from each participant and written consent was secured from a parent or legal guardian for participants under 18 years of age (Appendix D).

NOTE: This thesis represents part of a larger study that was entitled "The Importance of Dietary Intake of Beef on the Iron Status of Adolescents", which is stated on all of the above documents of approval. Intakes of beef, red meat and knowledge and attitudes about beef were also investigated and are reported elsewhere.

B. EXPERIMENTAL DESIGN

The present study was conducted at the University of Alberta, Department of Agricultural, Food and Nutritional Sciences. Two remote sites were utilized for data collection: McNally Composite High School and Ross Sheppard Composite High School, Edmonton, Alberta. The research was a cross-sectional survey design and utilized a convenience sample to assess adolescent iron status, dietary intake and health behaviours.

1. Participant Recruitment

Through contact with principals and teachers, several local high schools that indicated a willingness to participate in research were invited to participate in the research project. The two high schools that participated were selected on a "first come, first serve" basis. Within each school, science teachers were personally invited to allow their students to participate in the study and provide class time for data collection. A presentation was delivered at a regularly scheduled staff meeting outlining the research project, timelines for data collection and the time commitment required of teachers and students. Teachers were required to provide an entire teaching period (approximately one hour) to allow students to complete questionnaires during regular class time. In addition, teachers were required to allow the students to be absent from class (approximately 15 minutes) at a future date for blood collection. In return for this time, the teachers were offered a class on the role of iron in health to be delivered to their students by a member of the research team on a date following the completion of questionnaires.

The study was introduced to the students at the beginning of a regular class by the research coordinator. At this time, the research project was explained in detail, including the obligations of participants. Consent forms were distributed to all interested students and they were either signed and returned to the research coordinator, or were sent home with students under the age of 18 for parental consent. A letter of support from the school principal was also sent home attached to each consent form to inform parents of the school's approval of the research study. Consent forms were collected from students within a week to facilitate coding of questionnaire packages to maintain the anonymity of participants. Although a study of this nature cannot be truly anonymous because of feedback provided to participants, we felt student participation would be higher if confidentiality could be maintained regarding food and health questionnaires and blood samples. Each participant was assigned a code number and subsequently all material related to the research project identified the student by their code.

2. Description of Study Population

The total number of participants initially proposed for the study was approximately 390. The sample size calculation (Appendix E) determined a minimum of 195 participants would be required to detect significant differences between groups in terms of iron status. The eligibility criteria included students between the ages of 13 and 19. Otherwise, all students who expressed interest and provided consent were accepted to participate in the research study. An incentive was offered in the form of a draw for prizes upon completion of data collection.

C. METHODOLOGY

Data collection occurred at two separate time points. Data were collected from McNally Composite High School students during April and May 1998 and from Ross Sheppard Composite High School students during October and November 1998.

1. Anthropometric Measurements

Height was measured in the school on the day of blood sample collection. Participants removed their shoes, and height was determined to the nearest 0.1cm using a set square against a wall. Each participant was situated with their back against the wall with feet together and heels, buttocks and shoulder blades in contact with the wall. The set square was then lowered until it touched the top of the head and a mark was made on a piece of paper secured to the wall at the participant's height. Once the participant stepped away from the wall, height was determined using a tape measure.

Weight was measured to the nearest pound using a portable dial scale (Health o meter Professional Dial Scale, Model 150, Health o meter Inc., Bridgeview, Illinois). The scale was zeroed each morning and throughout the data collection period. Eventually all weights were converted to kilograms for the purpose of analysis. Body Mass Index (BMI) was calculated using the following equation:

$$\frac{\text{weight (kg)}}{\text{height (m)}^2}$$

The height and weight measurements for every participant were performed by the same member of the research team to minimize variation due to measurement error.

2. Biochemical and Hematologic Assessment of Iron Status

Two biochemical (serum ferritin, transferrin saturation) and one hematologic (hemoglobin concentration) variables were measured. Blood collection occurred at the respective schools during regularly scheduled classes within a time span of two to three days. Each blood draw was performed by the same trained technician (Dynacare-Kasper Medical Laboratories). A separate area (classroom, office) was provided for anthropometric assessment and blood collection. The blood collection area was further secluded from the waiting area to reduce the anxiety of participants.

Participants were retrieved from classes in groups of two or three and brought to the data collection room. Upon arrival, the research coordinator verified participant identification codes and provided each student with a requisition for the blood draw. Participants then proceeded one by one to the blood draw area where a 15mL sample of whole blood was drawn. On completion of the blood draw, students proceeded to the height/weight area for anthropometric assessment. Height, weight and blood collection took approximately 10 to 15 minutes and students were able to return to class afterwards. Cookies, donuts and juice were provided for participants once data collection was complete.

Blood draws were completed using one needle insertion. The multisample needle technique was employed with three vacutainers. The entire blood sample amounted to approximately 15mL, separated into three 5mL vacutainers. Each vacutainer was labeled with the participant's identification code to maintain confidentiality during blood analysis. Blood tubes were also labeled with the participant's date of birth as a second mode of identification. Throughout the blood collection period, blood tubes for hemoglobin were manually agitated to avoid coagulation. All samples were placed in a blood tube rack as they were collected. Blood samples were sealed in biohazard bags, placed in an appropriate container and transported in accordance with Transport of Dangerous Goods regulations to Dynacare Kasper Medical Laboratories for analysis. At this time, all three blood samples were centrifuged and analyzed. The maximum amount of time that passed before blood samples were centrifuged and analyzed was seven hours.

Serum ferritin

A 5mL blood sample for serum ferritin determination was collected in a serum separator tube (SST) to allow the blood to clot prior to centrifugation. Serum ferritin concentration was determined using the Chiron Diagnostics ACS:180® Automated Chemiluminescence System (Chiron Diagnostics Corporation, Norwood, MA, U.S.A.). Once blood samples are centrifuged and placed on the system, the system automatically dispenses 25µL of serum into a cuvette and dispenses 100µL of Lite Reagent and 450µL of Solid Phase into the sample. The sample is then incubated for 7.5 minutes at 37°C. The system then separates and aspirates the sample and dispenses 300µL of Reagent 1 and Reagent 2 to initiate the chemiluminescent reaction. The system then reports the result of the test, indicating the serum ferritin concentration of the sample. The reference range for serum ferritin is 12-1000µg/L (Dynacare Kasper Medical Laboratories, Edmonton, Alberta).

Transferrin saturation

A 5mL sample of blood was collected in a serum separator tube (SST) to allow sufficient clotting prior to centrifugation for the determination of transferrin saturation. Transferrin saturation was determined by first measuring total iron binding capacity and serum iron.

Serum iron and total iron binding capacity were determined colorimetrically and enzymatically using Sigma Diagnostics Iron and Total Iron-Binding Capacity reagents (Sigma Diagnostics, St. Louis, MO, U.S.A.).

Serum iron

Once blood samples were centrifuged, 0.5mL of serum was added to a cuvette containing 2.5mL Iron Buffer Reagent and mixed for serum iron determination. The absorbance of the sample was determined at 560nm using a RAXT calorimeter (Bayer, U.S.A.) and recorded as "INITIAL A". To the sample, 0.05mL of Iron Color Reagent was then added and the cuvette was mixed. The cuvette was then placed in a water bath at 37°C for 10 minutes. The absorbance of the sample was determined at 560nm using a spectrophotometer and recorded as "FINAL A". This process was duplicated in a cuvette labeled STANDARD that contained 0.5mL of Iron Standard instead of serum. A cuvette labelled BLANK was also utilized to be used as a reference in the spectrophotometer. To calculate serum iron, the following equations were used:

$$\Delta\text{ A TEST} = \text{FINAL } A_{\text{TEST}} - \text{INITIAL } A_{\text{TEST}}$$

$$\Delta\text{ A STANDARD} = \text{FINAL } A_{\text{STANDARD}} - \text{INITIAL } A_{\text{STANDARD}}$$

$$\text{Serum iron } (\mu g/dL) = \frac{\Delta A_{TEST}}{\Delta A_{STANDARD}} \times 500$$

TIBC

Once blood samples were centrifuged, 0.5mL of serum and 0.5mL Iron Standard were added to a cuvette containing 2.0mL UIBC Buffer Reagent and mixed for TIBC determination. The absorbance of the sample was determined at 560nm using a RAXT colorimeter (Bayer, U.S.A.) and recorded as "INITIAL A". To the sample, 0.05mL of Iron Color Reagent was then added and the cuvette was mixed. The cuvette was then placed in a water bath at 37°C for 10 minutes. The absorbance of the sample was determined at 560nm using a spectrophotometer and recorded as "FINAL A". This process was duplicated in a cuvette labeled STANDARD that contained 0.5mL iron free water and 0.5mL of Iron Standard instead of serum. A cuvette labelled BLANK was also utilized to be used as a reference in the spectrophotometer. To calculate serum unsaturated iron binding capacity (UIBC), the following equations were used:

$$\Delta \text{ A TEST} = \text{FINAL } A_{TEST} - \text{INITIAL } A_{TEST}$$

$$\Delta \text{ A STANDARD} = \text{FINAL } A_{STANDARD} - \text{INITIAL } A_{STANDARD}$$

$$\text{Serum UIBC } (\mu g/dL) = 500 - (\Delta A_{TEST} / \Delta A_{STANDARD} \times 500)$$

$$\text{Serum TIBC } (\mu g/dL) = \text{Serum Iron } (\mu g/dL) + \text{Serum UIBC } (\mu g/dL)$$

Transferrin saturation was calculated using the following equation:

$$\text{transferrin saturation } (\%) = \frac{\text{serum iron concentration } (\mu g/dL)}{\text{total iron binding capacity } (\mu g/dL)} \times 100$$

Transferrin saturation values between 16% and 60% are considered normal (Dynacare Kasper Medical Laboratories, Edmonton, Alberta).

Hemoglobin

A 5mL blood sample to assess hemoglobin concentration was collected in a vacutainer containing EDTA (anticoagulant). Hemoglobin concentration was determined using the Coulter STKS electronic counter (Coulter Electronics, Hialeah, FL, U.S.A.) and the Isoton 3 Reagent System. Hemoglobin values within the range of 120g/L to 160g/L for females and 130g/L to 160g/L for males are considered normal (Dynacare Kasper Medical Laboratories, Edmonton, Alberta).

Iron deficiency was defined as two or more indicators below normal. Iron deficiency anemia was defined as having all three parameters below normal. This criteria is similar to the approach used in the examination of the NHANES III (1988-1994) data (Looker et al., 1997). Table 3 depicts the multiple variable model used to determine the presence of iron deficiency and iron deficiency anemia.

Table 3: Multiple variable model for determination of iron deficiency and iron deficiency anemia

	NORMAL	DEPLETED IRON STORES	IRON DEFICIENCY	IRON DEFICIENCY ANEMIA
Serum ferritin	N	↓	↓	↓↓
Transferrin saturation	N	N	↓	↓
Hemoglobin	N	N	N	↓

Adapted from Yip & Dallman, 1996

At the completion of the data collection and analyses, all participants received a comprehensive summary of individual and group results, as well as general recommended dietary values and normal ranges for blood parameters that were measured (Appendix F). Participants were encouraged to contact the researcher for clarification of results or for answers to any questions related to the research project.

3. Assessment of Dietary Intake

Nutrient intake was obtained using a comprehensive, semiquantitative, 140 item FFQ (Appendix G). This information was collected in the school during a regularly scheduled class period. The research coordinator distributed the FFQ to participants and provided detailed verbal instructions regarding the use of the questionnaire. Standard sizes in relation to the portion sizes indicated in the FFQ were explained. Food models and sample containers were used to aid in the estimation of serving sizes. The students were also led through a sample FFQ to clarify the recording of information. Measures were taken to orient participants to the diet assessment period (i.e. past six months). To ensure consistent instruction, the research coordinator was present during every administration of the FFQ and responded to any questions that arose during the completion of the questionnaire. The FFQ took approximately 30 minutes to finish. Questionnaires were then collected and reviewed for errors and clarity of responses.

Prior to its administration, the original FFQ was modified to include foods that have appeared on the market since its inception. Also, an attempt was made to include foods that are commonly consumed by the adolescent population (ex. bagels, pizza snacks, orange drink, cappuccino) (Kushi, 1994). If students regularly consumed a food item that did not appear on the FFQ, they were encouraged to indicate names, portions and frequency of consumption of these foods to be included in the dietary analysis. Permission to use the original FFQ was granted by Dr. Bright-See of Brescia College, London, Ontario.

The FFQs were independently analyzed for dietary intake, excluding any vitamin or mineral supplements or herbal preparations. Analysis of the FFQs was performed by trained technical staff. Once entered, all diet data was independently verified for accuracy.

The FFQs were analyzed using a computerized nutrition software program (The Food Processor®, Version 6.0, ESHA Research, Salem, Oregon, U.S.A.). This software used a database of over 10, 000 foods from Canadian and United States Department of Agriculture databases and includes brand name items, fast foods and most commonly consumed items.

Dietary intake was quantified by creating a master food list that provided a template for each participant. The master food list was based directly on foods contained in the FFQ. Food codes were selected from the software data bank and weight values were assigned to each of the three allowable servings (small, medium, large). Individual participant responses were based on frequency of consumption over the previous six months. These responses were subsequently calculated into daily amounts. A serving size key was used to assist in coding the FFQ. The food lists were analyzed for energy (kcal/day), protein (g/day), carbohydrate (g/day), fat (g/day) and iron (mg/day) intake.

The proportion of dietary energy from of protein, carbohydrate and fat was also determined for females and males. Mean protein and carbohydrate intakes (in grams) were divided by total mean energy intakes (kcal) and multiplied by 4 (kcal/gram). Mean fat intakes (grams) were divided by total mean energy intakes and multiplied by 9 (kcal/gram). The adequacy of iron intake was determined by establishing a cutoff value of 2/3 of the RNI. Iron intakes less than 2/3 of the age and gender specific RNIs for iron (below 8.7mg/day and 6.7mg/day for females and males, respectively) were considered inadequate (Gibson, 1990b).

4. Assessment of Lifestyle Behaviours and Medical Factors

In order to assess potentially influential behaviours and medical status on iron nutriture, the General Health Information Questionnaire (Appendix H) was developed and validated. Prior to the present research, a study group was convened to develop the questionnaire and to identify relevant issues related to iron status based on the current literature. The study groups consisted of a Registered Dietitian/nutrition professor, a Registered Dietitian/graduate student and a fourth year nutrition student. Areas that were identified as the most pertinent to iron intake and status included current dietary practices, current exercise patterns, pertinent medical information, relevant pharmaceutical information, menstrual information and information regarding self-perceived health and lifestyle. These areas reflect the potential influence of lifestyle and medical factors and may help explain iron status.

Two focus groups were then conducted with two separate samples of teenagers from a local Girls and Boys Club to (1) refine and construct questionnaire items that targeted identified areas of interest and (2) assess the questionnaire for clarity of questions and content. The questionnaire was administered to the first focus group, which consisted of eight adolescents. Upon completion of the questionnaire, participants were asked for comments. Subsequently, inappropriate items were withdrawn or rewritten and the meanings of particular questions were explored and clarified. The questionnaire was administered to a second focus group consisting of eight adolescents. The additional comments obtained from the second focus group were used to further refine and clarify the questionnaire. At the same time, the questionnaire was reviewed by nutritionists and graduate students who had not been part of the original study group. Their suggestions were incorporated in the final revision of the questionnaire.

The revised General Health Information Questionnaire, as well as the FFQ were then pilot tested with a group of six adolescents recruited from the local community. Pilot testing was performed by the research coordinator to identify problems with questionnaire administration and to standardize the administration protocol.

In the larger study, the General Health Information Questionnaire was administered by the research coordinator during class time immediately following the FFQ. It took approximately 15 minutes for participants to complete.

The General Health Information Questionnaires were reviewed and scored by a group of trained nutritionists. All questionnaires were scored in a group setting to increase accuracy and consistency among reviewers. When discrepancies in interpretation arose, disagreement was resolved by consensus.

Most questionnaire items were examined categorically and were assessed in relation to iron status. Response categories included "Yes", "No" and "Sometimes" for most items. These responses were assigned the values of 1, 2, and 3, respectively for simplification of data entry and analysis. When items were answered "Sometimes" inappropriately (i.e. "Do you classify yourself as a vegetarian?"), responses were recorded as "Yes" or "No" based on responses to further questions (i.e. "Do you drink milk and/or eat other dairy products?"). Thus, participants were considered vegetarian if they were vegan, lacto vegetarian ovo vegetarian or lacto-ovo vegetarian. Physical activity was assessed based on exercise frequency and intensity. If participants indicated they exercised an average of 5 times a week or more, exercise intensity was assessed. If they were exercising at an intensity that would substantially increase heart rate (judged by the scorer), they were considered intense exercisers. Some items were assessed as continuous variables, such as the average number of cigarettes smoked per week.

Questionnaire results were independently verified for accuracy and entered into a spreadsheet (Microsoft® Excel 97) by the research coordinator.

D. CALIBRATION OF FOOD FREQUENCY QUESTIONNAIRE

Preliminary analysis of diet intake data as assessed with the FFQ revealed high mean intakes of energy and nutrients for several study participants, resulting in high group mean intakes for males and females. Subsequently, a calibration study was carried out to determine estimated "actual" daily intakes using three-day diet records. Thus, the relative validity of the FFQ in the adolescent population was assessed.

1. Ethical Approval

The calibration study received ethical approval from the University of Alberta. The Certificate of Approval from the Faculty of Agriculture, Forestry and Home Economics Human Ethics Review Committee is provided in Appendix I. Verbal approval was secured from the Associate Dean of Research, CAP, University of Alberta. Written, informed consent was obtained from participants as well as parents or legal guardians where appropriate (Appendix J).

2. Experimental Design

The calibration study was conducted at the University of Alberta, Department of Agricultural, Food and Nutritional Sciences. Data was collected at Ross Sheppard Composite High School, Edmonton, Alberta, approximately four months after participants had completed the FFQ for the larger study. The FFQs estimated usual diet intake retrospectively, over the previous six months, whereas the three-day diet records assessed intake prospectively. This design is advantageous as the completion of food records does not influence the participant's responses to the FFQ (Mares-Perlman et al., 1993).

Sixty-seven students from three classes were invited to participate in the calibration study. An incentive was offered to participants in the form of prizes for the 10 best diet intake records in terms of legibility, completeness and detail. Calibration studies are typically conducted on small samples relative to the larger study population. Thirty or 40 participants are considered adequate when assessing the dietary intake of a group in a study of this nature (Thompson & Byers, 1994).

3. Methodology

Estimated dietary intakes were obtained using a Diet Intake Record booklet (Appendix K). This information was collected independently by participants on their own time. Booklets were labeled with each participant's code number to maintain confidentiality of data.

During regularly scheduled class time, the research coordinator distributed booklets to participants and provided detailed verbal instructions regarding the use of the estimated diet intake record. Participants were instructed to record intake of all foods and beverages on the same three sequential days (Sunday, Monday and Tuesday) immediately following class instruction. Students were instructed to use measuring cups and spoons whenever possible to assist with portion size estimation. Suggestions were provided regarding portion estimation when measuring utensils were not available. Participants were also instructed to provide recipes for mixed dishes and labels for foods that were difficult to describe. Participants were encouraged to record food as soon as possible following ingestion. Students were led through a sample meal, demonstrating proper estimation and recording of information with appropriate detail. Questions were addressed at this time and the importance of accuracy, legibility and completeness of records was emphasized. Participants were encouraged to contact the research coordinator by telephone with any further questions throughout the recording period.

Diet intake record booklets were collected from participants the following week. Nutrient analysis of dietary intakes was performed by the research coordinator. Evaluation excluded vitamin or mineral supplements or herbal preparations. Dietary intakes were analyzed using Food Processor®, the same software used to analyze the FFQ. Foods and portion sizes were entered directly into the software program as they had been recorded in the diet intake record booklets. When the exact item could not be found in the database, an item with a similar nutrient profile was selected. Recipes were analyzed for nutrient content of individual portions prior to entry. Diet intake records were analyzed for energy (kcal/day), protein (g/day), carbohydrate (g/day), fat (g/day) and iron (mg/day) intake. As each diet intake record was assessed for nutrient content, results were transferred to a spreadsheet in Microsoft® Excel (1997). Intakes for the three days were averaged and these values were used for comparison with usual intakes as assessed by the FFQ.

E. STATISTICAL ANALYSES OF DATA

Statistical analyses were calculated using the software program Statistical Package for the Social Sciences (SPSS Version 8.0 for Windows). All data were entered into SPSS files from a spreadsheet in Microsoft® Excel (1997) within four months of data collection. Prior to analyses, all entries were verified comparing the recorded data to a printed copy of the SPSS data files. Results are presented as mean ± standard deviation, unless otherwise specified. All significant p-values ($p<0.05$) are indicated in bold print. Distribution of data was checked graphically and with descriptive statistics to assess normality (data not shown).

In order to test the hypotheses, the Independent Samples t-test (two-tailed) procedure was used to compare group means for all quantitative variables. Pearson's Chi-Square test (two-sided) was used to compare groups with categorical data. Fisher's Exact test (two-sided) was used to compare groups with categorical data with small *n*. Chi Square analysis was used to compare groups with low iron intakes (<RNI, <2/3 RNI) to groups with compromised iron status (iron deficiency, iron deficiency anemia). T-tests (for quantitative data) and Chi squared analyses (for categorical data) were used to compare iron intakes of vegetarians to non-vegetarians and dieters to non-dieters. Behavioural and medical factors were compared to iron status with Chi squared tests. Due to the low number of participants consuming inadequate amounts of iron and the low number of participants who had iron deficiency or iron deficiency anemia, the results of the Chi squared analyses showed no significant effect, therefore multiple linear regression and logistic regression could not be pursued.

Statistical analyses for the calibration study were calculated using SPSS Version 8.0 for Windows. All data were entered into SPSS files from a spreadsheet in Microsoft® Excel (1997) within four months of data collection. Results are presented as mean ± standard deviation. All significant p-values ($p<0.05$) are indicated in bold print. Distribution of data was checked graphically and with descriptive statistics to assess normality (data not shown).

The Paired Samples t-test (two-tailed) procedure was used to assess differences in energy, macronutrient and iron intakes estimated by the FFQ compared to average usual intakes estimated by the diet intake records for the participants of the calibration study. A significance level of $p<0.05$ was used to detect differences. Pearson correlation coefficient (r) was used to measure linear association between diet intake variables assessed by the two methods.

CHAPTER FOUR
RESULTS

1. Participant Recruitment

Potential participants were contacted through presentations given to high school science classes. Thirteen teachers volunteered 26 classes (approximately 25 students per class) for the opportunity to become involved in the project. Students from science, biology and physics classes were introduced to the study.

In total, 660 adolescents were invited to participate in the study. Four hundred and seven participants became involved in the data collection. Response rate varied among classes, the lowest being 20% and the highest 93%. Due to withdrawal and absence during data collection, the number of students who completed the study was 395, for a response rate of 60%.

Four hundred and seven adolescents completed questionnaires assessing dietary intake, lifestyle behaviours and medical history. Twelve FFQs were eliminated as they were completed incorrectly, resulting in a sample of 395 for questionnaire data. Three hundred and ninety-six adolescents were measured for height and weight and provided a blood sample for the determination of iron status (11 students who initially became involved in the study chose not to have blood drawn). The sample size goal of 390 (minimum sample size of 190 x 2, see Appendix E) was slightly exceeded, resulting in a final sample size of 395 participants. Given the survey nature of the study (incorporating nutrient intakes), the larger *n* provides a better estimate of usual dietary intake, as well as higher statistical power.

2. Participant Characteristics

Table 4 presents a summary of the demographic and anthropometric data for female and male participants. Variables are expressed as mean ± standard deviation. Ranges are also included where specified. There was no difference in average age or BMI between female and male adolescents. Males were significantly taller and heavier than females.

The BMI values demonstrate a wide range within each group. Seven female participants (3.0%) had a BMI of 29.4kg/m^2 or greater and nine male participants (5.5%) had a BMI of 29.6kg/m^2 or more. These participants are considered overweight according to age and gender specific cutoff values based on the recommendation of the Expert Committee on Clinical Guidelines for Overweight in Adolescent Preventive Services (Himes & Dietz, 1994).

Table 4: Demographic characteristics of adolescent study participants

VARIABLE	**OVERALL** *n = 407*	**FEMALES** *n = 241*	**MALES** *n = 166*	**p-value**[a]
Age (years)	16.0 ± 0.8[b] (14.0-19.0)[c]	16.0 ± 0.8 (14.0-19.0)	16.0 ± 0.8 (15.0-19.0)	0.959
Height (cm)[d]	168.4 ± 9.2 (148.2-199.0)	163.6 ± 7.4 (148.2-199.0)	175.2 ± 6.9 (158.0-191.6)	**0.001**
Weight (kg)[d]	61.8 ± 13.4 (39.0-122.9)	57.4 ± 11.3 (39.0-114.8)	68.0 ± 13.6 (44.5-122.9)	**0.001**
BMI (kg/m^2)[d]	21.7 ± 3.8 (15.0-43.0)	21.4 ± 3.7 (15.0-43.0)	22.0 ± 3.8 (17.0-38.0)	0.116

[a] Comparison of females to males
[b] Mean ± standard deviation
[c] Range
[d] *n = 396* (females *n = 233*, males *n = 163*)

There were no differences in height (p=0.73) and weight (p=0.14) between the group of participants measured in the spring and the group measured in the fall. Thus, all results are presented as combined data.

3. Iron Status

Table 5 presents the means, standard deviations and ranges of hematologic values for females and males. Average serum ferritin, transferrin saturation and hemoglobin concentration were significantly higher in male participants. Means of all measured parameters fell within normal ranges for each group.

Table 5: Laboratory parameters of adolescent study participants

VARIABLE	FEMALES *n = 232*	MALES *n = 164*	NORMAL RANGE	p-value[a]
Ferritin (μg/L)	31.7 ± 21.6[b] (2.0-130.0)[c]	62.1 ± 40.5 (10.0-277.0)	12-1000[d,e]	**0.001**
Transferrin Saturation (%)	27 ± 12 (2-73)	36 ± 12 (14-70)	16-60[d,e]	**0.001**
Hemoglobin (g/L)	133.4 ± 9.7 (75.0-152.0)	151.7 ± 8.6 (126.0-174.0)	120-160[d] 130-170[e]	**0.001**

[a] Comparison of females to males
[b] Mean ± standard deviation
[c] Range
[d] Females
[e] Males

Average ferritin was calculated for participants that had a history of recent infection and those with no recent infection. The mean ferritin did not differ between groups (p=0.066). For those with recent infection (n=292) mean ferritin was 42.3μg/L and for those with no recent infection (n=114) it was 49.3μg/L. The mean ferritin among females with a history of recent infection (n=179) was 31.0μg/L and for those with no recent infection (n=61) was 33.8μg/L. These groups demonstrated no difference in mean ferritin (p=0.392).

Table 6 (see next page) presents the number of participants who were below established cutoff values for the three parameters. Percent of each population is included in brackets. Compared to males, many more females were below cutoff values for all three measurements. Participants were considered iron deficient if two of the three measured parameters were below established gender-specific cutoff values. Several participants had only one parameter (serum ferritin, transferrin saturation, hemoglobin concentration) below cutoff values, as demonstrated in Table 6.

Table 6: Participants with biochemical measures below normal values[*]

MEASURE	**FEMALES** ***n (%)***	**MALES** ***n (%)***
Ferritin <12 µg/L	29 (12.5)[a]	1 (0.6)
Transferrin saturation <16%	32 (13.8)	4 (2.5)[b]
Hemoglobin <120 g/L	17 (7.3)	~
Hemoglobin <130 g/L	~	2 (1.2)

[*] p-values not available due to small *n* within groups
[a] Percentages determined from 232 females; 164 males
[b] Transferrin saturation n=163 due to one hemolyzed sample

Table 7 (see next page) illustrates the number of participants with iron deficiency using multiple criteria. The number and percent of participants who met the criteria for iron deficiency or iron deficiency anemia appears in bold print. In total, seven males had one of the three measured blood indices below cutoff values. No male participants met the criteria for iron deficiency (at least two of three parameters below cutoff values) or iron deficiency anemia (all three parameters below cutoff values). Fourteen female participants had iron deficiency and eight had iron deficiency anemia.

Table 7: Classification of iron deficiency using multiple criteria[*]

	FEMALES *n (%)*		**MALES** *n (%)*	
	Hb[a] <120 g/L	**Hb ≥120 g/L**	**Hb <130 g/L**	**Hb ≥130 g/L**
Ferritin <12 g/L	0	16 (6.9)[b]	0	1 (0.6)
T.S.[c] <16%	**1 (0.4)**	18 (7.8)	0	4 (2.4)
Ferritin <12 g/L + T.S. <16%	**8 (3.4)**	**5 (2.2)**	0	0
Ferritin ≥12 g/L + T.S. ≥16%	8 (3.4)	176 (75.9)	2 (1.2)	157 (95.7)

[*] p-values not available due to small *n* within groups
[a] Hb = hemoglobin
[b] Percentages determined from 232 females; 164 males
[c] T.S. = transferrin saturation

Table 8 presents the total number of participants who met the criteria for iron deficiency and iron deficiency anemia. The percent of each population appears in brackets. No males met the criteria for iron deficiency or iron deficiency anemia. Six percent (n=14) of the female participants were iron deficient and of these, 57% had iron deficiency anemia. The prevalence of iron deficiency anemia in the total female population was 3.4% (n=8).

Table 8: Prevalence of iron deficiency and iron deficiency anemia in adolescent study participants[*]

	FEMALES *n (%)*	**MALES** *n (%)*
Iron Deficiency (at least 2 of 3 parameters below normal)	14 (6.0)[a]	0
Iron Deficiency Anemia (all 3 parameters below normal)	8 (3.4)	0

[*] p-values not available due to small n within groups
[a] Percentages determined from 232 females; 164 males

The overall prevalence of iron deficiency in the total population was 3.5% (14 of 396). Due to the potential confounding of iron status variables in the presence of infection, further data analyses were performed. After excluding all participants that indicated a history of recent infection (n=292), the prevalence of iron deficiency in the remaining population was 0.1% higher than in the total population.

As recommended by Nordenberg et al., (1990) higher hemoglobin cutoff values were employed to diagnose iron deficiency among smokers. Hemoglobin cutoff values should be adjusted upward by 3g/L if 10-19 cigarettes are smoked/day, 5g/L if 20-39 cigarettes are smoked/day and 7g/L if 40 or more cigarettes are smoked/day. Fifty-two participants smoked cigarettes. Of those, eight participants smoked 10-19 cigarettes/day and no participants exceeded this rate of smoking. After adjusting cutoff values, no additional participants met the criteria for iron deficiency anemia. There was no difference in the prevalence of iron deficiency between the group of smokers and the group of non-smokers (p=0.688).

4. Dietary Intake

Upon preliminary analysis of energy and nutrient intakes of the study population, it was apparent some FFQs were completed inappropriately and indicated unrealistically high energy and nutrient intakes. A panel of nutrition researchers was consulted and 12 FFQs (less than 3% of questionnaires) were removed from the study based on consensus of the panel.

Table 9 summarizes the daily dietary intake of energy and selected nutrients for females and males. Variables are expressed as mean ± standard deviation. Ranges are included where specified. Males had significantly higher dietary intakes of energy and nutrients compared to females. The percent RNI was calculated to determine the extent to which participants were exceeding the RNI for iron.

Table 9: Average daily dietary intake of adolescent study participants

VARIABLE	**OVERALL** ***n = 395***	**FEMALES** ***n = 235***	**MALES** ***n = 160***	**p-value**[a]
Energy (kcal)	3221 ± 1386[b] (919-9204)[c]	2915 ±1124 (919-7140)	3671 ± 1599 (1128-9205)	**0.001**
Protein (grams)	128 ± 57 (32-400)	115 ± 45 (32-256)	146 ± 68 (40-400)	**0.001**
Carbohydrate (grams)	411 ± 178 (125-1136)	381 ± 157 (125-972)	455 ± 197 (128-1136)	**0.001**
Fat (grams)	124 ± 61 (24-388)	109 ± 50 (24-301)	146 ± 70 (37-388)	**0.001**
Iron (milligrams)	22.1 ± 10.5 (5.9-79.6)	20.5 ± 9.4 (5.9-54.0)	24.5 ± 11.6 (6.1-79.6)	**0.001**
IRON % RNI[d]	~	158[e]	245[f]	**0.001**

[a] Comparison of females to males
[b] Mean ± standard deviation
[c] Range
[d] Percent RNI (mean/RNI x 100, RNI = Recommended Nutrient Intake) (Health and Welfare Canada, 1990)
[e] RNI for females: 13mg/day (highest RNI for age range of participants)
[f] RNI for males: 10mg/day

The contribution of macronutrients to total energy was also calculated. The percent of energy among females from protein, carbohydrate and fat was 16%, 52% and 34%, respectively. Among males, the percent of energy from protein, carbohydrate and fat was 16%, 50% and 36%, respectively.

Table 10 compares females and males in terms of dietary adequacy of iron. The number and percent of teenagers in each group is presented. There were significantly more females obtaining less than the RNI for iron compared to males.

Table 10: Participants obtaining dietary iron below recommended levels

VARIABLE	**OVERALL** *n (%)*	**FEMALES** *n (%)*	**MALES** *n (%)*	**p-value**[a]
< RNI[b,c]	57 (14.4)[d]	47 (20.0)	10 (6.3)	**0.001**
< 2/3 RNI	8 (2.0)	6 (2.6)	2 (1.3)	*

[a] Comparison of females to males

[b] RNI for females: 13mg/day (highest RNI for age range of participants)(Health and Welfare Canada, 1990)

[c] RNI for males: 10mg/day

[d] Percentages determined from 395 overall; 235 females; 160 males

* p-value not available due to small *n* within groups

5. Dietary Behaviours

Prevalence of dieting, skipping meals and vegetarianism are illustrated in Table 11. The number of participants is indicated and the percent of each population appears in brackets. There was a significantly higher prevalence of dieting and meal skipping among females compared to males. There was no difference in prevalence of vegetarianism in females compared to males.

Table 11: Dietary behaviours of adolescent study participants

VARIABLE	**OVERALL** *n (%)*[b]	**FEMALES** *n (%)*	**MALES** *n (%)*	**p-value**[a]
Dieters (y/s)[c,d]	100 (24.6)[e]	80 (33.3)	20 (12.0)	**0.001**
Skip meals (y/s)[c]	244 (60.4)	154 (64.4)	90 (54.5)	**0.046**
Vegetarian[f]	9 (2.2)	7 (2.9)	2 (1.2)	0.320

[a] Comparison of females to males
[b] *n* may vary among variables due to respondent error
[c] Includes participant responses of "yes" and "sometimes"
[d] Participants were considered "dieters" if they were eating less to try to lose or maintain weight
[e] Percentages determined from 406 overall; 240 females; 166 males
[f] "Vegetarian" includes vegan, lacto-, ovo- and lacto-ovo-vegetarian participants

The group of dieters was compared to the group of non-dieters with respect to mean dietary iron intake. The prevalence of dieters and non-dieters achieving inadequate amounts of dietary iron was also analyzed. The results are presented in Table 12 (see next page). The mean iron intake was significantly higher in the group of non-dieters. As indicated in Table 12, the number of dieters and non-dieters who obtained inadequate amounts of dietary iron did not differ between the groups.

From the total sample, the mean daily energy intake of dieters was significantly less at 2788kcal than the non-dieters at 3364kcal (p=0.001). Among females, there was no difference between mean energy intakes of dieters versus non-dieters (2749kcal/day versus 2999kcal/day) (p=0.107).

Table 12: Comparison of iron intake among dieters and non-dieters within adolescent study population

DIETARY IRON	**DIETERS** *n = 98*	**NON-DIETERS** *n = 297*	**p-value**
Iron Intake (mg/day)[a]	20.2 ± 9.3	22.7 ± 10.8	**0.040**
< RNI[b,c]	17 (17.3)	40 (13.5)	0.343
< 2/3 RNI[b,c]	4 (4.1)	4 (1.3)	0.109

[a] Mean ± standard deviation
[b] Values reported as number of subjects, percent of subjects in parentheses
[c] RNI for females: 13mg/day, males: 10mg/day

Table 13 presents mean iron intake data for the group of vegetarian participants compared to the group of non-vegetarian participants. The number and percent of vegetarian and non-vegetarian participants who obtained inadequate amounts of dietary iron is also presented.

Table 13: Comparison of iron intake among vegetarian and non-vegetarian adolescent study participants

DIETARY IRON	**VEGETARIAN** *n = 9*	**NON-VEGETARIAN** *n = 386*	**p-value**
Iron Intake (mg/day)[a]	17.4 ± 7.5	22.2 ± 10.6	0.178
< RNI[b,c]	3 (33.3)	54 (14.0)	0.127
< 2/3 RNI[b,c]	2 (22.2)	6 (1.6)	**0.012**

[a] Mean ± standard deviation
[b] Values reported as number of subjects, percent of subjects in parentheses
[c] RNI for females: 13mg/day, males: 10mg/day

There was no difference in the amount of dietary iron obtained by vegetarians compared to non-vegetarians. There was no difference in the number of vegetarians and non-vegetarians consuming less then the RNI for iron. The number of non-vegetarians obtaining less than 2/3 of the RNI was significantly higher than the number of vegetarians.

The results were evaluated in terms of participants with inadequate iron intakes. Of the 57 participants achieving less than the RNI for iron, 17 were dieting and three were vegetarian. Of the dieters and vegetarians that were obtaining less then 13mg of iron per day, all of them were female. Although 10 males were consuming less than the RNI for iron (10mg/day), none of these males were dieting or consuming a vegetarian diet. Eight participants were consuming less than the 2/3 of the RNI for iron. Of these, six were female. Four of the females consuming less than 2/3 of the RNI for iron were dieting and two were consuming a vegetarian diet. Two males were obtaining less than 2/3 of the RNI for iron from their diets however, they were neither dieting nor vegetarian. Differences between female and male dieters and vegetarians could not be determined statistically due to insufficient numbers in these categories.

The iron status of male and female adolescents relative to inadequate dietary iron is presented in Table 14. The number and percent of deficient and anemic males and females obtaining less then the RNI is indicated.

Table 14: Iron status of adolescent study participants with inadequate iron intakes

VARIABLE	OVERALL *n = 395*[b]	FEMALES *n = 235*	MALES *n = 160*	p-value[a]
< RNI	57 (14.4)[c]	47 (20.0)	10 (6.3)	**0.001**
iron deficiency	0	0	0	*
iron deficiency anemia	1	1	0	*
< 2/3 RNI	8 (2.0)	6 (2.6)	2 (1.3)	*
iron deficiency	0	0	0	*
iron deficiency anemia	0	0	0	*

[a] Comparison of females to males
[b] *n* may vary among variables due to respondent error or withdrawal from study
[c] Values reported as number of subjects, percent of subjects in parentheses
* p-values not available due to small *n* within groups

There were significantly more females obtaining less then the RNI for iron compared to males. Fifty-seven of the participants who completed the study were consuming less than the RNI for iron. Of these, none were iron deficient and only one was classified as having iron deficiency anemia. Of the participants consuming less than 2/3 of the RNI for iron, none of them had a compromised iron status. No male participants were iron deficient or had iron deficiency anemia. Due to insufficient data, females and males could not be compared statistically for these variables.

6. Lifestyle Behaviours and Medical Factors

Table 15 presents prevalence of data for behavioural and medical factors that may influence iron status for male and female adolescents. The number and percent of females and males in each category is presented. Females and males were not different in terms of recent blood donation, cigarette smoking, incidence of recent infection, or previous diagnosis of anemia. Significantly more males were exercising intensely and significantly more females were using vitamin and mineral supplements. Thirty-four (14.5%) of the female participants had a heavy menstrual flow.

Table 15: Behavioural and medical factors of adolescent study participants

VARIABLE	**OVERALL** *n (%)*[b]	**FEMALES** *n (%)*	**MALES** *n (%)*	**p-value**[a]
Recent blood donation	14 (3.5)[c]	7 (2.9)	7 (4.2)	0.473
Intense exercise	85 (20.9)	35 (14.6)	50 (30.1)	**0.001**
Vitamin use	103 (25.6)	70 (29.4)	33 (20.0)	**0.033**
Smoke cigarettes	52 (12.8)	37 (15.4)	15 (9.0)	0.059
Recent infection	292 (72.1)	179 (74.6)	113 (68.1)	0.151
Previous dx[d] of anemia	20 (5.0)	16 (6.7)	4 (2.5)	0.054
Heavy menstrual flow	~	34 (14.5)	~	~

[a] Comparison of females to males
[b] *n* may vary among variables due to respondent error
[c] Percentages determined from 406 overall; 240 females; 166 males
[d] dx = diagnosis

Participants with compromised iron status (iron deficiency) were compared to participants with healthy iron status for behavioural and medical factors (Table 16). The number and percent of participants in each category is presented. As indicated in Table 16, no differences were observed between iron compromised and healthy iron status groups.

Table 16: Comparison of behavioural and medical factors of adolescent study participants with compromised iron status and healthy iron status

VARIABLE	**COMPROMISED IRON STATUS**[a] ***n = 14***	**HEALTHY IRON STATUS** ***n = 380***[b]	**p-value**
Recent blood donation	1 (7.1)[c]	12 (3.2)	0.380
Intense exercise	2 (14.3)	81 (21.3)	0.743
Vitamin use	1 (7.1)	96 (25.4)	0.204
Smoking cigarettes	2 (14.3)	47 (12.3)	0.688
Recent infection	10 (71.4)	273 (71.7)	0.985
Previous dx[d] **of anemia**	2 (14.3)	17 (4.5)	0.144

[a] Includes participants with at least 2 of 3 parameters below normal

[b] *n* may vary among variables due to respondent error, withdrawal from study or gender differences

[c] Values reported as number of subjects, percent of subjects in parentheses

[d] dx = diagnosis

7. Lifestyle Behaviours and Medical Factors Among Females

Iron deficiency and iron deficiency anemia appeared only in the female population. Table 17 (see next page) depicts the demographic, dietary, behavioural and medical information for female adolescents with compromised iron status and healthy iron status. Means and standard deviations are presented for numeric data and counts are presented for categorical data. There were no differences in any of the factors between the compromised and healthy iron status groups with the exception of iron intake. Participants with a compromised iron status consumed significantly less iron compared to female participants with healthy iron status. However, the mean iron intake for the iron compromised group was above the RNI for iron (13mg/day).

Table 17: Comparison of demographic, dietary, behavioural and medical factors among female participants with compromised iron status and healthy iron status

VARIABLE	COMPROMISED IRON STATUS[a] *n = 14*	HEALTHY IRON STATUS *n = 218*[b]	p-value
Age (years)[c]	16.3 ± 0.8	15.9 ± 0.8	0.118
Height (cm)[c]	161.4 ± 9.3	163.7 ± 7.3	0.248
Weight (kg)[c]	55.3 ± 7.7	57.6 ± 11.5	0.467
BMI (kg/m^2)[c]	21.2 ± 3.3	21.5 ± 3.8	0.813
Energy (kcal/day)[c]	2609 ± 635	2938 ± 1145	0.289
Iron (mg/day)[c]	16.4 ± 3.8	20.8 ± 9.6	**0.001**
Dieters (y/s)[d,e]	5	71	0.777
Skip meals (y/s)[d]	9	138	1.000
Vegetarian[f]	0	7	1.000
Recent blood donation	1	5	0.315
Intense exercise	2	32	1.000
Vitamin use	1	63	0.120
Smoke cigarettes	2	32	1.000
Recent infection	10	162	0.760
Previous dx[g] **of anemia**	2	13	0.229
Heavy menstrual flow	3	29	0.425
Number of years since menarche	3.2 ± 1.4	3.5 ± 1.4	0.491

[a] Includes participants with at least 2 of 3 indices of iron status below normal

[b] *n* may vary among variables due to respondent error or withdrawal from study

[c] Mean ± standard deviation

[d] Includes participant responses of "yes" and "sometimes"

[e] Participants were considered "dieters" if they were eating less to try to lose or maintain weight

[f] "Vegetarian" includes vegan, lacto-, ovo- and lacto-ovo-vegetarian participants

[g] dx = diagnosis

L

8. Calibration of Food Frequency Questionnaire

The relative validity of the FFQ was obtained by comparing dietary intake results with a three-day diet intake record in a sub-sample of the larger study population (Table 18). A self-selected population of 65 students (response rate of 97%) who participated in the larger study volunteered to participate. Eight estimated dietary intake records were discarded due to incomplete diet data or illegible information. Means and standard deviations are presented for each nutrient obtained with the two methods. There were no differences in dietary intake as assessed with the FFQ compared to the three-day record for energy, carbohydrate, fat and iron. The FFQ estimated a higher daily intake of protein. There was a strong ($p<0.01$) positive correlation for energy, carbohydrate and iron. There was a positive ($r=0.23$) association for fat. Despite a positive correlation for protein, the two methods measure protein intake differently.

Table 18: Calibration of the FFQ using a three-day estimated dietary intake record

VARIABLE	**FFQ** *n = 57*	**3-DAY RECORD** *n = 57*	**p-value (paired t-test)**	**Correlation Coefficients (r)**
Energy (kcal)	2776 ± 917[a]	2713 ± 906	0.645	0.355**
Protein (grams)	116 ± 48	102 ± 38	**0.035**	0.329*
Carbohydrate (grams)	364 ± 122	365 ± 121	0.922	0.469**
Fat (grams)	101 ± 40	94 ± 44	0.271	0.233
Iron (milligrams)	20.3 ± 8.8	18.7 ± 8.0	0.175	0.410**

[a] Mean ± standard deviation

* $p<0.05$

** $p<0.01$

9. Summary of Results with Reference to the Research Hypotheses

Hypothesis 1:

Adolescents with low dietary iron intakes (less than 2/3 of the RNI) will have iron deficiency or iron deficiency anemia.

This hypothesis was rejected as there were no adolescents with iron deficiency or iron deficiency anemia who were obtaining less then 2/3 of the RNI for iron from dietary sources as assessed with the FFQ.

Hypothesis 2:

Adolescents with low dietary iron intakes (less than 2/3 of the RNI) will be either dieting for the purposes of weight loss or weight maintenance or will be following a vegetarian diet.

This hypothesis was rejected as only 2% of the overall adolescent population was obtaining less than the 2/3 of the RNI for iron from dietary sources and of these, only half were dieting for weight loss or weight maintenance and only one fourth were consuming a vegetarian diet. There was no difference ($p=0.109$) in the number of participants obtaining less than 2/3 of the RNI for iron between the group of dieters and non-dieters. There were significantly more ($p=0.012$) non-vegetarians consuming less than 2/3 of the RNI for iron compared to vegetarians.

Hypothesis 3:

Behavioural factors such as recent blood donation, intense exercise, vitamin/mineral supplement use and smoking cigarettes will be predictive of iron status in adolescents.

This hypothesis was rejected as there were no significant differences ($p<0.05$) between the group of adolescents with compromised iron status and the group of adolescents with healthy iron status with respect to the above behavioural factors.

Hypothesis 4:

Medical factors such as recent infection, previous diagnosis of anemia, heavy menstrual flow and number of years since menarche in females will be predictive of iron status in adolescents.

This hypothesis was rejected as there were no significant differences ($p<0.05$) between the group of adolescents with compromised iron status and the group of adolescents with healthy iron status with respect to the above medical factors.

CHAPTER FIVE
DISCUSSION

A. MAJOR FINDINGS

Several findings have arisen from this research study. The major finding of this project was that the prevalence of iron deficiency was much lower than initially anticipated in female participants, based on similar research conducted in both the United States and Canada. The second major finding was that the average iron intake of the adolescents surveyed exceeded the Recommended Nutrient Intake for iron in both females and males. The third major finding was that the incidence of iron deficiency was not influenced by consuming a vegetarian diet or by restricting food intake for weight loss or weight maintenance. The fourth major finding was that the dietary, behavioural and medical factors thought to influence iron status demonstrated no difference between the iron deficient group and the group of participants with a healthy iron status, with the exception of dietary iron intake. The final major finding of this research study was that, although modified from its original form, the FFQ utilized in the present study was a valid tool for estimating the usual dietary intake of adolescents, as demonstrated through a calibration study. Overall, these results suggest that the population of adolescents surveyed had a healthy iron status relative to other similar groups previously assessed and also had an adequate iron intake, above recommended levels.

B. DISCUSSION

1. Participant Characteristics

Students that became involved in the research study were from a middle socioeconomic level. Although parental and family income was not obtained through socio-demographic questionnaires, teachers postulated students generally were from this economic classification. The study sample was obtained from and approximately representative of a larger multi-racial/ethnic adolescent population. The ethnic diversity of the sample was not assessed through questionnaires but appeared to be representative of the students attending the schools and the population of students of this geographic area and economic level. The study population was primarily Caucasian, and based on the author's observation non-participants did not appear to be any different than participants.

In the total sample population, males were taller and heavier than females. The two groups did not differ in age or BMI. In comparison with the most recent reference data compiled by Rosner and colleagues (1998), participant BMIs in the present study were similar to those of the adolescent population of the same age and ethnicity surveyed in nine large national and state epidemiological studies in the United States. The mean and standard deviation for BMI for White American 16 year old females was 21.9 ± 4.0 and for males was 22.1± 3.8 (Rosner et al., 1998) compared to 21.4 ± 3.7 for females and 22.0 ± 3.8 for males in the present study. Data collection for the total sample occurred at two separate time points. Approximately half of the participants were surveyed in the spring and half were surveyed the following fall. The two data collection time points did not indicate seasonal variation in participant growth as the group of participants surveyed in the spring was not different from the group surveyed in the fall in terms of height and weight ($p=0.73$ and $p=0.14$, respectively).

The Expert Committee on Clinical Guidelines for Overweight in Adolescent Preventive Services (Himes & Dietz, 1994) recommends using ≥95th percentile for age and gender or >30 (kg/m^2), whichever is smaller, to classify overweight. Adolescents with a body mass index ≥85th percentile and less than the 95th percentile or ≤30 (kg/m^2) should be considered at risk for overweight (Himes & Dietz, 1994). According to this classification, seven (3.0 %) female participants and 9 (5.5%) male participants from the present study were overweight and 21 (9.1%) females and 15 (9.1%) males were at risk for overweight. The prevalence of overweight in the present sample was considerably lower among females compared to approximately 6.3% in U.S. adolescent girls of the same age and ethnicity (Rosner et al., 1998). The prevalence of overweight among males was more comparable to U.S. adolescent boys at approximately 6.7% (Rosner et al., 1998).

2. Iron Status

Distinct gender differences were observed in all hematologic measurements. Mean serum ferritin of males was almost double that of females, and mean transferrin saturation and hemoglobin concentration were significantly higher in males compared to females ($p=0.001$). Mean values for all measurements were within normal ranges.

The proportion of females with hematologic values below established cutoffs exceeded that of males in all iron status measurements. For example, 12.5% of females had ferritin <12µg/L compared to less than 1% of males below this cutoff. The observed gender differences were expected and have previously been well documented (Samuelson et al., 1996; Hallberg et al., 1993; Seoane et al., 1985; Bothwell et al., 1979). Given that serum ferritin is a direct reflection of body iron stores (Bothwell et al., 1979; Cook & Finch, 1979; Lipschitz et al., 1974), these results indicate that the demand for iron among females compromises their ability to establish iron stores the way adolescent boys can. These findings reinforce the vulnerability of the female adolescent population. Menstruation imposes a regular depletion of iron reserves and places a woman at high risk for anemia in the event of pregnancy. Participants with low iron reserves may not adequately meet a physiological challenge to their iron stores.

While low serum ferritin values indicate impaired iron stores, high values do not necessarily indicate increased iron stores, as ferritin can be increased by several factors including infection. Within this population, the effects of recent infection were expected to have the most profound influence on iron status. However, this was not substantiated by the data, as the mean serum ferritin concentration was similar in those who indicated a history of recent infection and those who indicated no recent infection.

Iron deficiency was non-existent in the 164 males assessed in the present study. Although seven males did fall below the established cutoff values for serum ferritin, transferrin saturation and hemoglobin concentration, none of them met the multiple criteria necessary for the determination of iron deficiency. The prevalence of iron deficiency among females was 6%, an incidence that is lower than the most recent estimates among U.S. adolescents. The prevalence of iron deficiency in the U.S. was estimated by Looker and colleagues (1997) and is based on data from the third National Health and Nutrition Examination Survey (NAHNES III), conducted between 1988 and

1994. Using similar hematologic measurements and diagnostic criteria, these researchers estimated the prevalence of iron deficiency to be 9% in females between 12 and 15 years of age and 11% in females between 16 and 19 years of age. The prevalence among males of the same age categories was estimated at ≤1% (Looker et al., 1997).

Compared to two other Canadian studies of iron status in adolescents, the prevalence of iron deficiency in the present study is considerably lower. Valberg and colleagues (1976) estimated a high probability of iron deficiency in 30% of their adolescent population of 204. The estimated probability among females was 40% as interpolated from a published figure (Valberg et al., 1976). These results were based solely on the determination of iron deficiency characterized by low body iron stores, or serum ferritin below 15ng/mL. Research conducted on the iron status of 574 Canadian adolescents by Seoane and colleagues (1985) indicated the prevalence of iron deficiency was 39% in the total population and 35% in the female population. Again, this determination was made based on serum ferritin less than 12ng/mL.

Several factors may influence the validity of comparing the prevalence of iron deficiency in different groups examined by different researchers. Such comparisons are difficult because of a lack of consensus on the biochemical criteria used for the determination of iron deficiency and iron deficiency anemia and the cutoff values used for hematologic parameters. The multiple variable model used to determine the presence of iron deficiency in this study is similar to that used by Looker and colleagues (1997). There are several advantages to using this technique, the main one being the increased accuracy of detecting iron deficiency. It has been established that "no single biochemical indicator currently available is consistently diagnostic of iron deficiency" (Expert Scientific Working Group, 1985, p.1320). The use of any of the relevant hematologic measurements in isolation has the disadvantage of low specificity and sensitivity for iron deficiency. Serum ferritin, although the most sensitive measure of the early stages of iron depletion, can be influenced by inflammation, infection, hyperthyroidism and diseases that elicit damage to organs and tissues (Hallberg, 1995; Lipschits et al., 1974). Transferrin saturation is useful in detecting the stage of iron deficiency when iron stores are low enough to influence the functional iron compartment. Low transferrin saturation in combination with low serum ferritin indicates advanced iron deficiency (Herbert, 1992). Hemoglobin has low sensitivity, as it does not fall until the late stages iron deficiency. It is a useful measurement for detecting iron deficiency anemia, but demonstrates no observable change in mild iron deficiency.

Generally, the presence of two or more abnormal values measuring iron status is indicative of impaired iron status (Gibson, 1990a; Expert Scientific Working Group, 1985). The combined use of serum ferritin with other measures provides better accuracy in detecting the stage of iron deficiency and offsets the inadequacies of each measurement used in isolation (Pilon et al., 1981).

The cutoff value of 12μg/L for serum ferritin is the generally accepted lower limit for adults and is commonly used in adolescents (Pilch & Senti, 1984). Transferrin saturation below 16% indicates iron deficient erythropoesis in adults (Dallman, 1977). Cutoff values ranging from 12% to 16% have been used when assessing the prevalence of iron deficiency in children aged one to 14 (Pilch & Senti, 1984) to account for age-related differences in normal serum iron (but not TIBC) (Gibson, 1990a). However, the transferrin saturation cutoff value of 16% has been used in one other Canadian study (Valberg et al., 1976) and was selected for the present study as a conservative cutoff point to detect impaired iron status using the multiple variable model technique. The age and gender-specific cutoff values for hemoglobin concentration are based on the World Health Organization Technical Report on Nutritional Anemia (1972). Concentrations of hemoglobin below 120g/L and 130g/L in females and males, respectively, over age 14 are most likely associated with the presence of anemia (WHO, 1972). The same hemoglobin values are recommended by Dallman (1977) as they represent the lower limit for normal hemoglobin for females and males between the ages of 12 and 18. Reference ranges for the above parameters are derived statistically from epidemiological data based on the distribution of values in healthy populations (Gibson, 1990a).

The prevalence of iron deficiency anemia in the present study is comparable to estimates in other similar populations in Canada, the United States and Sweden, ranging from 0% to 3% (Looker et al., 1997; Sameulson et al., 1996; Valberg et al., 1976).

3. Dietary Intake

Assessment of dietary intake in this study revealed significant gender differences between group means of all measured parameters (p=0.001). Adolescent males had 20% to 35% higher mean intakes of macronutrients compared to females. Iron intake was 20% higher among males compared to females. These gender differences were expected and similar findings have been reported in other studies (Shatenstein & Ghadirian, 1996; Briefel et al., 1995; Gibbons et al., 1995). The relatively low average nutrient intakes of females is attributed to their significantly lower food consumption. On average, males ingested 25% more calories than females in this study. Pao and Mickle (1981) reported that the intake of most nutrients increases proportionately with energy intakes. Therefore, it should be expected that the male adolescents would have higher intakes of all measured dietary parameters as their energy intakes were higher than females.

In comparison to other research studies that estimated energy consumption of adolescents utilizing a FFQ, the present population had similar intakes. Feunekes and colleagues (1998) recently assessed the intakes of 15 year old girls and boys (n=587) in The Netherlands and found the mean energy intake was 2652kcal/day among female and 3633kcal/day among male participants. A Canadian nutrition survey conducted in Manitoba utilized a FFQ and indicated the mean intake of females was 2280kcal/day and males was 3092kcal (Sevenhuysen et al., 1993). Although the age range in the Manitoba study was slightly higher than the present population (18-34 years), the geographic locale and food intake measurement instrument are similar to the present study. An American study conducted by Salz and colleagues (1983) utilized a one-day food intake recall and assessed the energy intakes of females and males between 15 and 19 years of age. Adolescent females (n=263) had a mean daily intake of 2020kcal and males (n=287) had a mean daily intake of 3243kcal. The estimation of energy intake of adolescents appears to be highly inconsistent between studies, and the variability of measurement techniques limits the comparability of research. However, the mean energy intakes in the present study (females, 2915kcal/day & males 3671kcal/day) are not unlike similar studies and reflect usual food consumption patterns, as indicated by the calibration study.

Female and male groups had mean iron intakes that exceeded the RNI (Health and Welfare Canada, 1990) for iron. When group means for iron were calculated as a percent of the RNI, females were consuming 158% and males 245%. Several researchers have documented male iron intakes in excess of recommended levels, however, average female iron intakes are often inadequate and fall below recommendations (U.S. Department of Agriculture, 1997; Devaney et al., 1995; Gibbons et al., 1995; Bull & Phil, 1992; Absolon et al., 1988; Valberg et al., 1976). Other studies within Canada indicate that dietary intake of iron is insufficient among a high proportion of adolescent females (Absolon et al., 1988; Valberg, 1976). The present female population is unique in that the mean iron intake greatly exceeded the RNI for iron.

The high dietary iron intakes could be explained in part by the fact that participants were of middle socioeconomic level. It could be assumed that these adolescents would have access to healthy foods, providing good variety and high quality sources of iron.

Nutrient supplements can provide substantial amounts of selected nutrients for some individuals. Attempts were made to evaluate the use of vitamin and mineral supplements on two questionnaires: the FFQ and the General Health Information Questionnaire. Due to the uncertainty of many supplement users as to what their supplements contained and the variety of supplements available, supplements were not included in the evaluation of nutrient intake. Vitamin/mineral supplements were used by 26% of the present population and their contribution to overall nutrient intakes is unknown.

The FFQ was selected as the diet assessment technique in the present study because it is the best method available for assessing usual intakes in large groups in terms of cost and convenience. Also, it places the focus on habitual food intake and circumvents recent changes in diet by obtaining information about dietary intake over a previous time period (Thompson & Byers, 1994). Another advantage to using the FFQ is that it reduces the response bias that can be inherent due to the lower response rates associated with more rigorous and time-consuming diet assessment methods. Furthermore, dietary intake methods that assess intake over shorter periods of time (such as the 24 hour recall method) cannot account for the high day-to-day variability of food consumption. The use of the FFQ in this study was particularly appropriate as iron status can be considered as the balance between iron intake and loss over preceding weeks and months (Preziosi et al., 1994). The retrospective nature of the FFQ provided nutrient intake data appropriate for investigating relationships between diet and iron status.

The results of the FFQ and other dietary assessment techniques are dependent upon the motivation, compliance and ability of participants to accurately report usual food intake (Torun et al., 1996). Participants in the present study demonstrated motivation and compliance by completing FFQs within the allotted time period. Only 12 FFQs were filled out incorrectly and subsequently were eliminated. Participants were strongly encouraged to consider usual food intake over the previous six months when answering diet intake questions. The FFQ used in the present study was self-administered, however the research coordinator was present throughout questionnaire administration which provided several advantages. First, the presence of an "interviewer" is more likely to maintain a participant's interest. Second, areas of respondent misunderstanding can be clarified consistently among participants. Third, amounts of food can be quantified with more detail using food models or other explanations and examples. Fourth, instructions for completing the FFQ can be delivered uniformly to all participants (Jain et al., 1996). These factors likely reduced respondent and interviewer bias to an appreciable extent.

Zulkifli and Yu (1992) indicated that the FFQ can overestimate usual food intake. According to Lui (1994), the systematic bias contained in a FFQ with a long list of food items and food groups can cause overestimation of nutrient intake. Consequently, the relative validity of the FFQ used in the present study was assessed with a calibration study. The results verify the FFQ was a valid measurement tool within the present population as intakes assessed using the FFQ were not different than intakes assessed using a three-day diet intake record. Based on the calibration study, intakes did not appear to be overestimated by the FFQ.

The categorization of iron intakes less than 2/3 of recommended levels as inadequate have been used in several other studies (Seiga-Riz et al., 1998; Zive et al., 1996; Anderson et al., 1982). Gibson (1990b) states that using 2/3 RNI as the cutoff value reduces the tendency to overestimate the actual incidence of inadequate intakes. Individuals consuming nutrients below 2/3 RNI can more accurately be classified as "at risk" for inadequate intakes. In this study, only eight individuals had an iron intake below 2/3 of the RNI. None of the participants consuming less than 2/3 of the RNI had iron deficiency or iron deficiency anemia. These findings indicate that factors other than total dietary iron intake influence iron status.

4. Dietary Behaviours

In the present research, dietary behaviours encompassed dieting, vegetarianism and meal skipping. There were significantly more females dieting and skipping meals compared to adolescent males ($p<0.05$). More females were eating a vegetarian diet, although the difference was not significant ($p=0.320$). It was expected that these three behaviours would be more prevalent among females compared to males as a means of dieting for weight control.

It has previously been documented that female adolescents have more weight and dieting concerns, are more critical toward their body and tend to correct imperfections through their diet (Casper & Offer, 1990; Wardle & Beales, 1986; Clifford, 1971). Similar concerns and behaviours are relatively uncommon in male adolescents (Neumark-Sztainer et al., 1997; Casper & Offer, 1990; Huenemann et al., 1966). A recent provincial survey conducted in British Columbia (The McCreary Centre Society Adolescent Health Survey, 1993) revealed female adolescents are more likely to view themselves as overweight compared to males (43% vs. 20%). Moreover, 22% of females were "not satisfied" with their body weight compared to 10% of males, whereas 41% of males were "very satisfied" with their body weight compared to only 25% of females (The McCreary Centre Society Adolescent Health Survey, 1993).

A recent study conducted by Field and colleagues (1999b) estimated 32% of approximately 8600 preadolescent and adolescent girls surveyed (aged nine to 14 years) indicated they were currently trying to lose weight. The prevalence of trying to lose weight among approximately 7500 boys of the same age in the same study was only 20% (Field et al., 1999b). In the Youth Risk Behaviour Survey of U.S. teenagers in grades nine through 12, approximately 44% of females were trying to lose weight compared to only 15% of males (Center for Disease Control, 1991). In British Columbia, 68% of adolescent females surveyed were actively trying to lose or maintain weight in the seven days prior to evaluation whereas only 32% of males were (The McCreary Centre Society Adolescent Health Survey, 1993).

The gender difference in dieting in the present study indicates that girls were four times as likely as boys to diet. This is not as high as estimates by Rosen and Gross (1987), whose research demonstrated high school girls were five times more likely to diet compared to their male peers. The prevalence of dieting (eating less to try and lose or maintain weight) among females in the present study was 33%, which is similar to findings in other adolescent populations. Other studies that assessed dieting in teenage girls have estimated prevalence rates ranging from 13% in a Canadian survey (The McCreary Centre Society Adolescent Health Survey, 1993) to 21% (Neumark-Sztainer et al., 1997), 24% (Absolon et al., 1988), 37% (Dwyer et al., 1967) and 65% (Rosen & Gross, 1987) in the United States. Although the prevalence of teenage girls engaging in diet restriction in the present study is high compared to some research, it is not considered abnormally high for adolescent females in general.

In this study, it was hypothesized that adolescents with dietary intakes of iron less than 2/3 of the RNI would be either dieting or practicing vegetarianism. Of the eight participants in this study consuming less than 2/3 of the RNI for iron, four were also dieting. Looking at it from another perspective, among the 98 dieters, only four (4%) were consuming a diet that supplied less than 2/3 of the RNI for iron. Ninety-six percent of the dieters were obtaining adequate iron (>2/3 RNI). The number of participants consuming less than 2/3 of the RNI for iron did not differ between the group of dieters versus the group of non-dieters (p=0.109). This result indicates that adequate amounts of dietary iron can be obtained when attempting to lose or maintain weight. In terms of dietary iron intake, the mean iron intake of dieters was significantly lower than the group of non-dieters (p=0.040), however the mean intakes of both groups exceeded the RNI. These results indicate that although significantly less dietary iron was obtained by dieters compared to non-dieters, the average amount consumed was above recommended levels in the adolescents surveyed. Overall, the energy restriction of dieters was modest (mean=2788kcal). In the absence of a more severe energy restriction, reduced iron intakes would not be expected. Although the FFQ was retrospective in nature and the General Health Information Questionnaire assessed current dieting practices, the tendency with FFQs is to report recent food intake, thereby reflecting more recent intakes for comparison to current dieting practices.

Vegetarianism was more prevalent in teenage girls in this study, compared to boys, although the difference was not significant (p=0.320). Individuals adopt a vegetarian style of eating for many reasons including ethical, moral, and health reasons. A vegetarian diet may also be embraced as a means of weight control or weight loss, a means that is more socially acceptable than calorie restriction, especially in the female adolescent population (Szabo, 1997). In any case, the vegetarian diet presents unique nutrition concerns in adolescents, a population with elevated nutrition requirements to meet the demands of growth. Adolescent girls, with the onset of menstruation, have an increased need for iron and without heme sources from the diet may be unable to get adequate iron from dietary sources. There were no vegans in the present study, hence a vegetarian was defined as an individual who excluded meat, excluded meat and milk or meat and eggs.

The prevalence of vegetarianism was relatively low in this study, 2% of the total population and approximately 3% of females. Seven of the nine vegetarians (78%) were female. These results are comparable to a recent study conducted in the U.S. Less than 1% of the adolescents in the Minnesota Adolescent Health Survey identified themselves as vegetarian (Nuemark-Sztainer et al., 1997). Of the 107 vegetarians, 81% were female (Nuemark-Sztainer et al., 1997). The prevalence of vegetarianism (those who do not consume red meat) in South Australia occurred in 8 to 10% of teenage girls and 1 to 2% of teenage boys (Worsley & Skrzypiec, 1998).

One of the hypotheses in this study was that adolescents with dietary intakes less than 2/3 of the RNI for iron would be dieting or eating a vegetarian diet. Only eight participants were consuming less than 2/3 of the RNI for iron. Of those eight, only two were vegetarian. Looking more specifically at the total population, the prevalence of vegetarianism was very low. Nine participants in this study indicated they consume a vegetarian diet. Of the vegetarians, only two (22%) were obtaining inadequate quantities of iron (<2/3 RNI) from the diet. In terms of dietary adequacy, these results are comparable to a study conducted in female adolescents in Ontario where 26% of lacto-ovo vegetarians were consuming less than 2/3 of the RNI for iron (Donovan & Gibson, 1996).

There were nine participants consuming a vegetarian diet in the present study. Seven of the vegetarians were obtaining adequate dietary iron. In the comparison of vegetarians to non-vegetarians, the average iron intake did not differ between groups (p=0.178) and the mean intake of iron in both groups exceeded the RNI. Significantly more non-vegetarians were obtaining less than 2/3 of the RNI for iron compared to vegetarians (p=0.012). This finding suggests that a vegetarian diet can provide adequate iron and the probability of consuming inadequate amounts of dietary iron was not related to consuming a vegetarian diet within this adolescent population.

Meal skipping occurred in 60% of the adolescents surveyed. The incidence was higher in teenage girls compared to boys (64% and 55%, respectively). It has previously been documented that teenage girls use this behaviour as a weight loss technique (Gibbons et al., 1995; Rosen & Gross, 1987). This relationship was not directly addressed in the present study. However, it was initially speculated that skipping meals may reflect busy lifestyles, and influence overall diet quality and thus have an impact on iron status. This assumption was not substantiated with the data analyses.

5. Lifestyle Behaviours and Medical Factors

Several behaviours and medical factors that can potentially influence iron status were selected and assessed in relation to iron status among adolescents. In comparing females to males, the only measures that were different between groups were intense exercise and use of vitamin supplements (p<0.05). There were no differences between groups with respect to recent blood donation, smoking cigarettes, recent infection, or previous diagnosis of anemia.

In this study, more males were engaging in intense exercise compared to females. The higher level of activity in adolescent males in the present study is similar to the results of the British Columbia survey, which found regular exercise more common in teenage males (The McCreary Centre Society Adolescent Health Survey, 1993). A similar gender disparity was seen in adolescents across Canada. The proportion of physically active males between the ages of 15 and 19 exceeded that of females (approximately 43% versus 24%, respectively as interpolated from a published chart) (Statistics Canada, 1995). The higher occurrence of vitamin and mineral supplement use among female adolescents in this adolescent population (29% in females compared to 20% in males) may have demonstrated greater differences in nutrient intakes between the two groups. However, the overall contribution of vitamin and mineral supplements to the diet was not assessed.

Lifestyle behaviours and medical factors were assessed comparing the group of participants with compromised iron status to the group of participants with healthy iron status. There were no differences between these two groups in any of the parameters measured. These results indicate that the incidence of these lifestyle behaviours and medical factors were no more common among iron deficient individuals when compared to healthy individuals.

Ideally, regression analysis is the best statistical technique to determine relationships of this nature. Logistic regression can be used to predict the presence or absence of an outcome (i.e. iron deficiency) based on predictor variables (i.e. lifestyle behaviours and medical factors). This method is appropriate for use when dependent variables are dichotomous. Linear regression can be used to determine the dependent variable (i.e. dieting or vegetarianism) that best predicts the independent variable (i.e. iron deficiency) (Daniel, 1995). However, due to the low number of iron deficient participants (n=14), regression analysis could not be utilized.

6. Lifestyle Behaviours and Medical Factors Among Females

In the present study, iron deficiency occurred only in females. Hence, the group of female participants was divided into two groups, those with compromised iron status and those with healthy iron status. These two groups were then compared for demographic measurements, dietary intake, dietary behaviours, lifestyle behaviours and medical factors. There were no differences between the two groups in any of the variables, with the exception of dietary iron intake. Adolescent females with healthy iron status were consuming more iron than those with compromised iron status ($p<0.05$). However, both groups were consuming dietary iron in excess of recommended levels. These results indicate potentially influential demographic variables, dietary and lifestyle behaviours and medical factors were no more prevalent in adolescent females with iron deficiency than in healthy adolescent females. These results also indicate that the only difference between the two groups of female adolescents was mean iron intake. The results suggest, but cannot firmly establish, the type of dietary iron consumed could be a factor influencing iron status.

A dietary constituent that was not evaluated was the source of dietary iron. The diet analysis software used (The Food Processor®, Version 6.0, ESHA Research, Salem, Oregon, U.S.A.) did not distinguish between the two types of dietary iron. If measured, the proportion of heme versus non-heme iron in the diet may have elucidated a potential influence of dietary iron type in these two groups. It has been illustrated previously that the both the quantity and quality of dietary iron are determining factors in meeting iron requirements (Salas et al., 1990). Although the group of females with compromised iron status were consuming iron in excess of the RNI, it may have been mainly non-heme iron with low bioavailability.

7. Calibration of Food Frequency Questionnaire

The calibration study demonstrated that the FFQ was a valid tool for assessment of nutrient intake in the adolescent population. It has previously been established that the self-administered FFQ provides an accurate assessment of adolescents' dietary intakes (Rockett et al., 1997). However, Lui (1994) recommended a calibration study should be conducted to evaluate the FFQ within the population of interest. The true validity of reported food intakes can be impractical, difficult and expensive when using observation techniques or biochemical markers, such as doubly labeled water. Rather than measure the absolute validity of dietary intake, the relative validity can be measured by evaluating the results obtained using a "test" method against those obtained using a "reference" method (Gibson, 1990).

The relative validity of the FFQ used in the present study was assessed by comparison with a three day recorded estimate of food intake in a sub-sample of the larger population. Although random days are preferable for optimum reliability (Larkin et al., 1991), a three day sequential record was selected in the present study for practical reasons. The diet intake record was completed approximately four months after participants completed FFQs for the larger study. The design of the calibration study was such that the completion of the food records *after* dietary intakes were evaluated using the FFQ would not influence participant responses on the FFQ.

C. LIMITATIONS OF THE STUDY

The main limitation of the study was that the sample size was inadequate to generate a significant sub-group of adolescents with compromised iron status. A greater number of individuals with iron deficiency and iron deficiency anemia is necessary to demonstrate associations between dietary, lifestyle and medical factors and iron status. Furthermore, since the study was a cross-sectional design, inferences as to causality can not be drawn. Instead, only probable associations between behaviours and outcomes can be established. Another limitation of this study lies within the sample population. The adolescents evaluated were apparently healthy and within this geographic area, health care is generally accessible and affordable. Thus, it was not surprising to find no influence of medical factors on iron status. In addition, the socioeconomic level of participants places them in a lower risk category compared to teenagers below or near the poverty level. The generalizability of the results is limited. However, the present sample can be regarded as a reference population group for comparison in other Canadian studies. A methodological limitation of the study was the incapability of the diet analysis software to determine the source and type of dietary iron. This data may have provided more detailed information as to the difference in iron intake among iron compromised and iron replete females, despite mean iron intakes above recommended levels. In addition, more detailed information regarding vitamin and mineral supplement intakes (such as brand name and supplement contents) may have provided a better dietary profile of study participants. Finally, a sample bias may have been introduced by inviting primarily science students to participate in the study. Although this was advantageous in terms of participant recruitment, the characteristics of science students may not be typical of the larger population of adolescents in this socioeconomic level and geographic locale.

In summary, the relatively low prevalence of iron deficiency among adolescents, and more specifically among adolescent females was somewhat unexpected. Factors contributing to the low prevalence of impaired iron status may be related to the high socioeconomic level of participants and the subsequent availability of a varied diet containing iron that is highly bioavailable. Other factors include adequate iron intakes despite dieting among females, the low prevalence of vegetarianism and the absence of extreme behavioural or medical factors that significantly impacted on iron status.

CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

A. CONCLUSION

Previous research has indicated that adolescents, particularly adolescent females, comprise a group that is at high risk for compromised iron status. The present study does not support these findings. In contrast, it clearly demonstrates that the adolescents assessed were at low risk for iron deficiency and iron deficiency anemia, as indicated through the low prevalence of these conditions. In addition, a relationship between inadequate dietary iron intake and iron deficiency was not substantiated as none of the participants with compromised iron status were consuming iron in quantities below 2/3 of the RNI. This finding is in contrast to the assertion by Marx (1997) that iron deficiency in developed countries frequently occurs due to low iron intake. The research also revealed no association between dietary iron intake and dietary behaviours. A relationship between inadequate dietary iron intake and dieting was not established by the research findings as there was no difference between the group of dieters and the group of non-dieters in terms of the proportion of adolescents obtaining less than 2/3 of the RNI for iron. Also, a relationship between inadequate dietary iron intake and vegetarianism was not established as there were significantly more participants obtaining less than 2/3 of the RNI for iron who were consuming a non-vegetarian diet. In contrast to the original hypothesis, these results indicate the probability of obtaining an inadequate quantity of dietary iron may be higher when consuming a non-vegetarian diet within the population studied. The lack of association between the dietary, behavioural and medical factors measured indicates these elements elicit no influence on iron status, with the exception of females. In the total population, the prevalence of iron deficiency was sufficiently low that relationships or lack of relationships could not be firmly established. Factors postulated to influence iron status included recent blood donation, intense exercise, vitamin/mineral supplement use, recent infection and previous diagnosis of anemia (and heavy menstrual flow and years since menarche in females). There was no evidence in this adolescent population that iron status was influenced by any of these factors. When analyzed separately, dietary iron intake was significantly lower among iron deficient teenage girls. Although the mean iron intake of this group was above the RNI for iron, perhaps iron absorption was sub-optimal. Several factors in addition to the total iron content of the diet determine overall iron bioavailability. The type of iron consumed as well as other dietary constituents affect the efficiency of iron absorption and may contribute to poor iron status. This consideration should be accounted for in future studies. Within the total sample of adolescents studied, there were many differences between gender groups. However, almost no differences existed between the iron compromised and iron replete groups.

B. FUTURE RESEARCH

Based on the research findings, it is apparent that not all adolescents are at high risk for iron deficiency. The population assessed generally had a healthy iron status, with a low prevalence of iron deficiency. Future research of this nature should determine which groups within the adolescent population are at high risk for iron deficiency and develop strategies to reduce risk in these population subgroups.

In order to obtain a clearer association of the dietary components that influence iron nutriture, several factors should be considered. These include dietary sources of iron, dietary inhibitors and enhancers of iron absorption and the influence of vitamin and mineral supplementation. When assessing dietary intakes in relation to iron status, iron bioavailability should be assessed in future studies.

The behavioural and medical factors predictive of iron status could not be determined in this study. Future research should target an iron deficient population to elucidate the effects of these potential influences on iron status.

Despite the myriad of factors influencing food choices, including social and cultural norms and values, stressful lifestyles, peer and media influence and personal and sociocultural expectations of appearance, teenagers were eating adequate amounts of nutrients. This could indicate a current shift in adolescents' perceptions of health and food choices, a shift towards healthier eating. This is a possibility that merits further investigation within this cohort. Perhaps it is time for health care providers to evaluate current health-related behaviours among teenagers and reframe the message to fit the present needs and behaviours of adolescents. In order to maximize program effectiveness, adolescents should be involved in the development of health promotion efforts and be allowed to identify their health priorities.

This is the only recent study of this magnitude in Canada assessing nutritional intakes and iron status of a population of adolescents. While most health professionals would concede that nutrition and health in youth is an important issue, there is limited current research on the dietary choices, nutritional status and health and lifestyle behaviours in adolescents. Consequently, there is a need for further research within this population.

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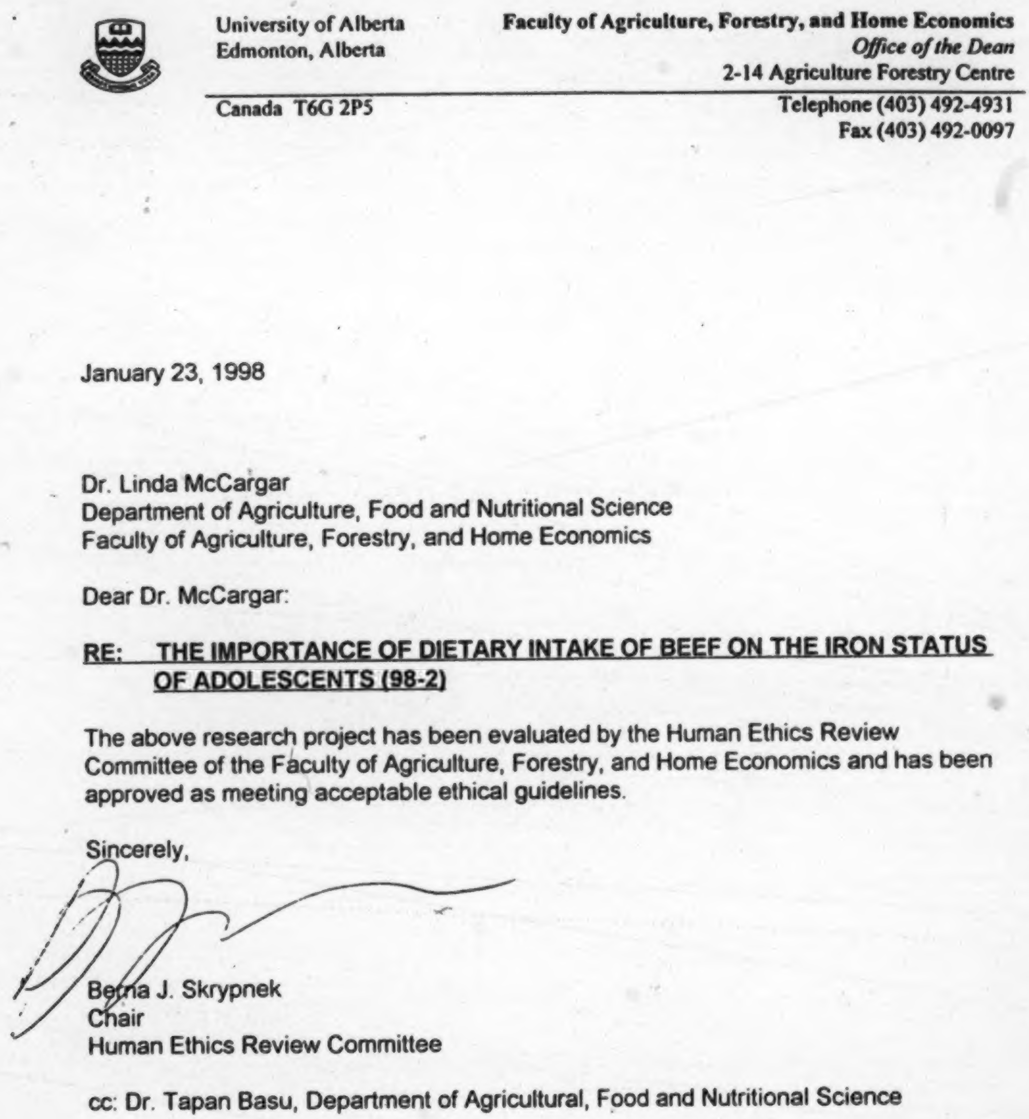
APPENDIX A: Approval – Human Ethics Review Committee



University of Alberta
Edmonton, Alberta
Canada T6G 2P5

Faculty of Agriculture, Forestry, and Home Economics
Office of the Dean
2-14 Agriculture Forestry Centre
Telephone (403) 492-4931
Fax (403) 492-0097

January 23, 1998

Dr. Linda McCargar
Department of Agriculture, Food and Nutritional Science
Faculty of Agriculture, Forestry, and Home Economics

Dear Dr. McCargar:

RE: THE IMPORTANCE OF DIETARY INTAKE OF BEEF ON THE IRON STATUS OF ADOLESCENTS (98-2)

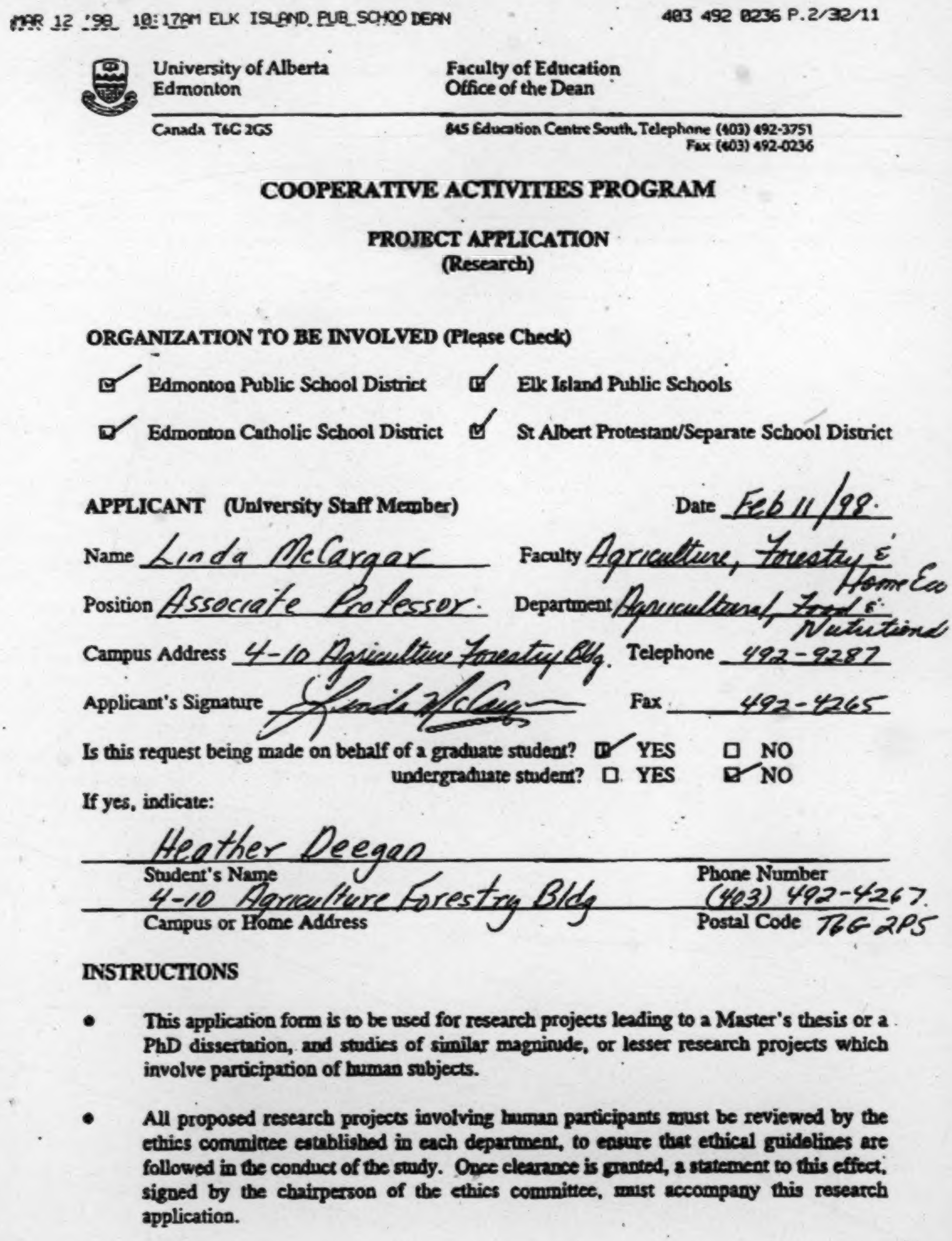
The above research project has been evaluated by the Human Ethics Review Committee of the Faculty of Agriculture, Forestry, and Home Economics and has been approved as meeting acceptable ethical guidelines.

Sincerely,

Berna J. Skrypnek
Chair
Human Ethics Review Committee

cc: Dr. Tapan Basu, Department of Agricultural, Food and Nutritional Science

APPENDIX B: Approval – Cooperative Activities Program (CAP)



MAR 12 '98 10:17AM ELK ISLAND PUB SCHOO DEAN 403 492 0236 P.2/32/11

University of Alberta
Edmonton

Faculty of Education
Office of the Dean

Canada T6G 2G5

845 Education Centre South, Telephone (403) 492-3751
Fax (403) 492-0236

COOPERATIVE ACTIVITIES PROGRAM

PROJECT APPLICATION
(Research)

ORGANIZATION TO BE INVOLVED (Please Check)

☑ Edmonton Public School District ☑ Elk Island Public Schools

☑ Edmonton Catholic School District ☑ St Albert Protestant/Separate School District

APPLICANT (University Staff Member) Date Feb 11/98.

Name Linda McCargar Faculty Agriculture, Forestry & Home Eco

Position Associate Professor. Department Agricultural, Food & Nutritional

Campus Address 4-10 Agriculture Forestry Bldg. Telephone 492-9287

Applicant's Signature Linda McCargar Fax 492-4265

Is this request being made on behalf of a graduate student? ☑ YES ☐ NO

undergraduate student? ☐ YES ☑ NO

If yes, indicate:

Heather Deegan
Student's Name

Phone Number
(403) 492-4267.

4-10 Agriculture Forestry Bldg
Campus or Home Address

Postal Code T6G 2P5

INSTRUCTIONS

- This application form is to be used for research projects leading to a Master's thesis or a PhD dissertation, and studies of similar magnitude, or lesser research projects which involve participation of human subjects.
- All proposed research projects involving human participants must be reviewed by the ethics committee established in each department, to ensure that ethical guidelines are followed in the conduct of the study. Once clearance is granted, a statement to this effect, signed by the chairperson of the ethics committee, must accompany this research application.

Attn: Linda McCargar & Heather Deegan

-2-

- **TYPEWRITTEN** responses to the following four descriptors are required. Submit this form and appropriate attachments to Assistant Dean, Research (845 Education South).

1. **Description of Research Project.** Include title, objectives, procedure, evaluation instruments, ethical considerations, etc. If appropriate, include parental/guardian consent forms, copy of survey/questionnaire if used, etc.

2. **Description of how this activity is of value to the school(s) or school district(s) involved.** Include perceived benefits to school, students, teachers, administrators, district.

3. **Suggested personnel, school and times.** Specify anticipated duration and number of visits.

4. **Anticipated project timeline and completion date of final report.**

Note:
Gene has not signed this one?
Terry.

CE USE ONLY:

y Grace Malicky, Assoc Dean, Research Date March 10, 1998
Grace Malicky

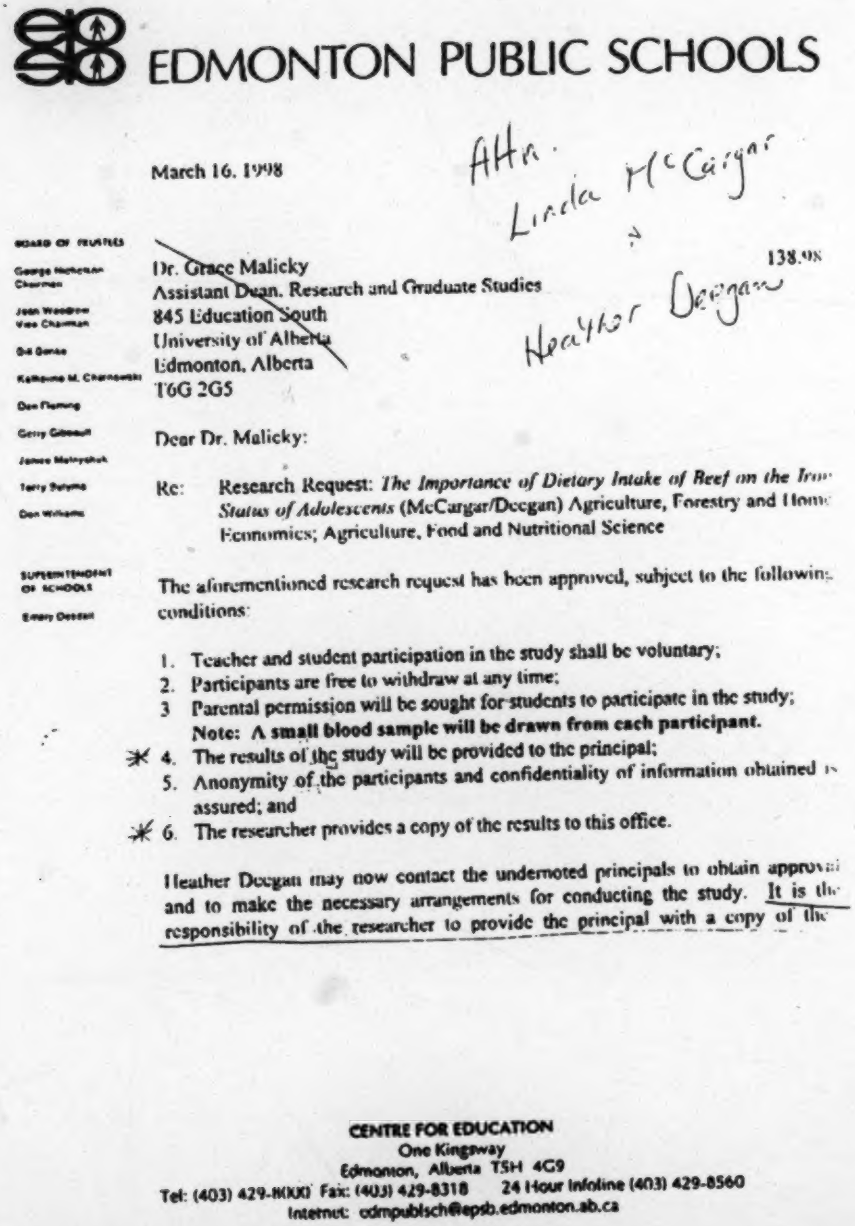
Approved by Gene, Superintendent Date March 10/98
Position

Subject to the following condition: The results of the project findings are to be forwarded to the cooperating school(s) and school district's central office. ☑ YES ☐ NO

The Importance of Dietary Intake, etc.

1997.06.24
\exp\applicat

APPENDIX C: Approval – Edmonton Public Schools



EDMONTON PUBLIC SCHOOLS

March 16, 1998

Attn.
Linda McCargar
&
Heather Deegan

138.98

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Emery Dosdall

Dr. Grace Malicky
Assistant Dean, Research and Graduate Studies
845 Education South
University of Alberta
Edmonton, Alberta
T6G 2G5

Dear Dr. Malicky:

Re: Research Request: *The Importance of Dietary Intake of Beef on the Iro Status of Adolescents* (McCargar/Deegan) Agriculture, Forestry and Hom Economics; Agriculture, Food and Nutritional Science

The aforementioned research request has been approved, subject to the followin conditions:

1. Teacher and student participation in the study shall be voluntary;
2. Participants are free to withdraw at any time;
3 Parental permission will be sought for students to participate in the study;
   Note: A small blood sample will be drawn from each participant.

* 4. The results of the study will be provided to the principal;
5. Anonymity of the participants and confidentiality of information obtained is assured; and

* 6. The researcher provides a copy of the results to this office.

Heather Deegan may now contact the undernoted principals to obtain approv and to make the necessary arrangements for conducting the study. It is the responsibility of the researcher to provide the principal with a copy of the

CENTRE FOR EDUCATION
One Kingsway
Edmonton, Alberta T5H 4G9
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proposal and all related documents. I wish Heather success in this endeavour an anticipate reception of the results as they become available. If you require furthe information, please contact Jane Kinoshita at 429-8232.

Sincerely,

Jane Kinoshita
Research Liaison, Consulting Services

JK/bl

cc: Maureen Dean, principal, Bonnie Doon School
George Rice, principal, McNally School
Ed Butler, principal, Parkdale School
Ron Fortin, principal, Ross Sheppard School
Bob Maskell, principal, Victoria School

APPENDIX D: Consent Form

INFORMATION SHEET

TITLE OF RESEARCH PROJECT: **The Importance of Dietary Intake of Beef in the Iron Status of Adolescent**

INVESTIGATOR(S):	Linda McCargar PhD RD	492-9287
	Tapan Basu PhD	492-7921
	Heather Deegan BASc RD	492-4267

Background: Iron deficiency is the most common micro-nutrient deficiency in the world. Adolescents have been identified as a group that is at increased risk. The requirement for iron is elevated during the teenage years and dietary intake of iron-rich foods is often low. However, it is not known why iron intake is low in this population. This study will attempt to determine if low iron status in adolescents is associated with low red meat consumption. Also, it will assess why individuals choose not to consume red meat. Answering these questions will help us understand some reasons behind the increased risk of iron deficiency in adolescents and whether or not this risk could be improved by increasing knowledge about dietary sources of iron.

Purpose: The purpose of this study is to investigate the influence of red meat consumption on the iron status of 13-19 year old males and females. We will also assess teenagers' knowledge, attitudes and perceptions about red meat in their diets.

Procedures: This study will involve three parts, all of which will be completed in school:

Part I - Questionnaires
The research team will briefly introduce the study and administer three questionnaires to students.

1) A Food Frequency Questionnaire (FFQ): Requires the participant to estimate the frequency of consumption of specified food items.

2) General Health Information Questionnaire: Assesses dietary habits and general health.

3) Knowledge and Attitudes about Beef: Assesses knowledge and attitudes about red meat.

The administration of the questionnaires will take approximately **45 minutes**.

Part II – Blood Work
An 8 mL blood sample will be drawn from each participant to be analyzed for serum ferritin (a measure of iron stores in the body), transferrin saturation (a measure of iron in the blood), and hemoglobin (a protein in the blood made of iron). Trained medical laboratory technicians from Dynacare Kasper Medical Laboratories will perform all blood draws using standard sterile procedures.
This will take approximately **15 minutes per student**.

Part III – Feedback & Results
The research team will return to the classroom to provide each student with the results of their diet analysis and results of their blood work. Preliminary results will also be presented allowing students to see how their iron intake and iron status compares with adolescents across Canada and with other students who participated in this study.

Possible Benefits: Each student will find out what their iron status is and whether their diet is providing adequate iron. They will learn something relevant to their own health and will also learn how to eat better to improve their health. This research project has been made available to the students to reinforce some of the concepts introduced in their classes and also allows them to get involved in active scientific research in progress. We will collect information required to develop education strategies aimed at increasing the consumption of iron-rich foods in adolescents, which ultimately will help improve the iron status of the adolescent population as a whole.

Possible Risks: The potential risks are minimal. There should be no discomfort or adverse effects to the students participating in this study. Blood tests may result in minor pain and possible bruising at the time of the blood draw. Qualified, trained lab personnel from Dynacare Kasper Medical Laboratories will take the blood samples under all circumstances. If abnormal results are found in any of the procedures listed above, this information will be shared with the participant. At that time, she/he will be directed to consult with the appropriate medical personnel (ie. his/her family doctor) for verification.

Confidentiality: Personal records relating to this study will be kept strictly confidential. The participant's name will not be attached to any of the data sheets or blood work and results will be shared only with the individual participant. Code numbers will be used rather than names on all documents and all files will be stored in a locked file cabinet. Any report published as a result of this study will present group results only and no individual will be identified.

Compensation: An incentive for participation will be offered in the form of a draw for prizes after every one-hundred participants have provided the required data. There will be four draws in total and the chances of winning are approximately 1 in 10.

Time Commitment: The total time commitment required is approximately 1 hour.

Results: Each participant will have the opportunity to review their results with the researcher.

Funding Agency: This research is funded by The Beef Information Centre which obtains its funding from the Beef Industry Development Fund. The Beef Information Centre's main goal is to increase public awareness about beef.

Withdrawal From Study: You are free to withdraw from this research study at any time without jeopardy. You will be promptly informed if any knowledge gained from this or any other study becomes available which could influence your decision to continue in the study. Under all circumstances, the information gathered from this study will be communicated only to individual participants in an open and confidential manner.

Any Questions? Please contact:

Heather Deegan RD, MSc Candidate
Department of Agricultural, Food and
Nutritional Science
Phone number: 492-4267

-OR-

Linda McCargar PhD RD, Associate Professor
Department of Agricultural, Food and
Nutritional Science
Phone number: 492-9287

Consent Form

I acknowledge that the research procedures described on the Information Sheet (attached), of which I have a copy, are clear to me. Any questions I may have had were answered to my satisfaction. In addition, I know that I may contact the persons designated on this form if I have further questions either now or in the future. I understand the possible benefits of joining the research study. I also understand the possible risks and discomforts. I have been assured that personal records relating to this study will be kept confidential. I understand that I am free to withdraw from the study at any time without jeopardy to myself. I understand that if any knowledge gained from the study is forthcoming that could influence my decision to continue in this study, I will be promptly informed.

Individuals who may be
contacted about the research are:

Heather Deegan BASc RD
Research Co-ordinator
Agricultural, Food &
Nutritional Science
Phone: 492-4267

-OR-

Linda McCargar PhD RD
Associate Professor
Agricultural, Food &
Nutritional Science
Phone: 492-9287

____
(Name of Participant)

____
(Signature of Participant) *must be obtained first

____
(Name of parent or legal guardian of participants under the age of 18)

____
(Signature of parent or legal guardian of participants under the age of 18)

____
(Date)

____
(Name of Witness)

____
(Signature of Witness)

____
(Signature of Investigator)

APPENDIX E: Sample Size Calculation

Estimation of sample size for independent groups was conducted using the hemoglobin concentration (continuous variable) of iron deficient males and females as the end-point. Previous research conducted by Hallberg and colleagues (1993) assessed the prevalence of iron deficiency in adolescents and identified iron deficient individuals according to serum ferritin values (Hallberg et al., 1993). Means and standard deviations are based on the results of Hallberg et al (1993). Calculations are based on a 95% confidence interval and a power of 90% using a two-sided test.

The appropriate calculations for the determination of sample size follow:

$$n = \frac{(SD_1^2 + SD_2^2)(Z_{1-\beta} + Z_{1-\alpha/2})^2}{(X_2 - X_1)^2}$$

$$n = \frac{(9.8^2 + 8.5^2)(1.28 + 1.96)^2}{(142 - 132)^2}$$

$$n = \frac{(168.3)(10.5)}{100}$$

$$n = \frac{1767.2}{100}$$

$$n = 17.7$$

According to recent estimates of iron deficiency in the United States, the expected prevalence of iron deficiency in the present sample is estimated to be 11% among females (Looker et al., 1997). Thus, a sample of ~195 (17.7 x 11) would be the minimum sample size required to detect a similar prevalence of iron deficiency in adolescent females. The minimum sample size was doubled for a final sample size goal of 390 female and male adolescents.

Due to the overwhelming response of participants and the survey nature of the study (incorporating dietary intakes), the original sample size goal was slightly exceeded. The result is a higher statistical power (>90%), as well as a larger sample population, which may better represent the nutrient intakes of the adolescent population.

Method from Cheney & Boushey, 1992

APPENDIX F: Participant Results Package

Results Package

Name:

Thank you for completing the Iron Study. If you would like to discuss your results you can contact me at 492-4267.

Heather Deegan, RD, BASc.
University of Alberta

General Information

Gender	Male	Female	Overall
Total Number	166	237	221
Average Age	16	16	16

Blood Analysis

Blood	Individual	Normal Range Male	Normal Range Female	Average Male	Average Female
Ferritin (ug/L)	8	12-1000	12-1000	61	32
Hemoglobin (g/L)	129	130-170	120-160	152	133
Saturation Index	0.19	[0.16-0.60]	[0.16-0.60]	0.36	0.27

Diet Analysis

Diet	Individual	Average Male	Average Female	Average Overall	Average North American Teenager	Recommended Nutrient Intake
Protein (%)	10.20	15.1	14.7	14.9	13.9	13-15% of total kcal
Fat (%)	29.20	36.0	33.6	34.8	32.9	30% of total kcal
Carbohydrate (%)	62.41	50.3	53.4	51.9	53.4	50-60% of total kcal
Iron (mg)	10.6	29.8	24.3	26.5	13-20	Female 13mg Male 10mg

APPENDIX G: Food Frequency Questionnaire

Food Frequency Questionnaire

FOOD INTAKE

This questionnaire measures your **usual** food intake **over the last 6 months**. Answer the questions on the following pages, including food and beverages you take both at home and away from home.

For every food, mark "Yes" or "No". If "Yes", indicate the number of times per day, week or month. Then mark one of the serving sizes. If "No", mark it and go on to the next question.

BEOFRE YOU GET STARTED, CHECK OUT SOME EXAMPLES!

EXAMPLES:

1. "Sarah drinks 1% milk once a day – about 1 ½ cups each time."
This is how she would record her milk.

	Do you eat or drink this at least once a month?	**How many times per day or week or month?**		**about how much do you have each time?**		
1% milk and beverages made with it	☐ Yes → ☐ No	___	☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ 1 cup	☐ more than 1 cup

2. "Sarah uses whole wheat bread in her sandwiches for lunch about five times a week, two slices each time."
She would record her bread this way.

Whole wheat or light rye bread and rolls	☐ Yes → ☐ No	___	☐ Day ☐ Week ☐ Month	☐ 1-2 slices	☐ 3-4 slices	☐ 5 or more slices

3. "Sarah eats roast beef or steak every 3 or 4 months."
She would record her beef like this.

Beef and steak roasted or stewed	☐ Yes → ☐ No	___	☐ Day ☐ Week ☐ Month	☐ 4 ounces	☐ more than 4 ounces	☐ less than 4 ounces

Now…start at the top of the next page.
Please read the items carefully and take your time answering the questions.

ID #

		Do you eat or drink this at least once a month?	How many times per day or week or month?		about how much do you have each time?		
	• **White or Chocolate Milk to drink (NOT including milk on cereal)**						
1.	Skim milk and beverages made with it	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ 1 cup	☐ more than 1 cup
2.	1% milk and beverages made with it	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ 1 cup	☐ more than 1 cup
3.	2% milk and beverages made with it	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ 1 cup	☐ more than 1 cup
4.	Whole milk and beverages made with it	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ 1 cup	☐ more than 1 cup
5.	Meal replacement drinks (Ensure, Boost, Carnation Instant Breakfast)	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ 1 can (235 mL)	☐ less than 1 can	☐ more than 1 can
6.	Milkshakes	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ 1 cup	☐ more than 1 cup
	• **Cheese, Yogurt and Eggs**						
7.	Skim milk cheese such as low fat mozzarella	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ 1 in. cube	☐ more than 1 in. cube	☐ less than 1 in. cube
8.	Hard cheese such as cheddar, Swiss	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ 1 inch cube	☐ more than 1 inch cube	☐ less than 1 inch cube
9.	Processed cheese slices (including on sandwiches and burgers)	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ 1 slice	☐ 2 slices	☐ more than 2 slices
10.	Low fat cottage cheese	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ more than ½ cup	☐ less than ½ cup

		Do you eat or drink this at least once a month?	**How many times per day or week or month?**	**about how much do you have each time?**		
11.	Cottage cheese	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ more than ½ cup	☐ less than ½ cup
12.	Processed cheese spreads (Cheez Whiz, Philadelphia Cream Cheese)	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 Tbsp.	☐ more than 1 Tbsp.	☐ less than 1 Tbsp.
13.	Low fat yogurt	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ small tub	☐ large tub	
14.	Regular yogurt	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ small tub	☐ large tub	
15.	Eggs	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 egg	☐ 2 eggs	☐ 3 or more eggs
	• **Breakfast cereals**					
16.	Whole grain hot cereals (rolled oats, Red River)	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ¾ cup	☐ more than ¾ cup	☐ less than ¾ cup
17.	Instant hot cereals (Cream of Wheat)	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ¾ cup	☐ more than ¾ cup	☐ less than ¾ cup
18.	Sweetened instant hot cereals (flavored Quaker oats)	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ¾ cup	☐ more than ¾ cup	☐ less than ¾ cup
19.	Bran-type cold cereals (Bran Flakes, All Bran, Raisin Bran)	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ¾ cup	☐ more than ¾ cup	☐ less than ¾ cup
20.	Unsweetened cold cereals (Cheerios, Rice Krispies, Corn Flakes, Shredded Wheat)	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ¾ cup	☐ more than ¾ cup	☐ less than ¾ cup

		Do you eat or drink this at least once a month?	**How many times per day or week or month?**	**about how much do you have each time?**		
21.	Sweetened cold cereals (Honey Nut Cheerios, Mini Wheats, Honey Combs, Frosted Flakes)	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ¾ cup	☐ more than ¾ cup	☐ less than ¾ cup
22.	Granola cereal	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ¾ cup	☐ more than ¾ cup	☐ less than ¾ cup

23. If you eat cereal:

a) Do you usually add sugar? ☐ Yes ☐ No

b) Do you usually add artificial sweetener? ☐ Yes ☐ No

c) Which one of the following do you usually use on your cereal? (circle one)

Half & Half Cream Whole Milk 2% Milk 1% Milk Skim Milk

d) How much milk do you add to your cereal? ☐ ½ cup ☐ more than ½ cup ☐ less than ½ cup

		Do you eat or drink this at least once a month?	**How many times per day or week or month?**	**about how much do you have each time?**		
	• **Breads, Rolls and Muffins**					
24.	Whole wheat or light rye bread and rolls	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1-2 slices	☐ 3-4 slices	☐ 5 or more slices
25.	Dark rye, pumpernickel, high-fibre bread and rolls	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1-2 slices	☐ 3-4 slices	☐ 5 or more slices
26.	White, Italian, French, egg, cheese, raisin bread and rolls	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1-2 slices	☐ 3-4 slices	☐ 5 or more slices

		Do you eat or drink this at least once a month?	How many times per day or week or month?		about how much do you have each time?		
27.	Bagels, hot dog or hamburger buns	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ ½	☐ 1	☐ 2 or more
28.	Plain or low fat crackers (soda, melba toast, bread sticks, Snack Well's)	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ 1-5	☐ 6-10	☐ more than 10
29.	Crackers (Ritz or cheese-type)	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ 1-5	☐ 6-10	☐ more than 10
30.	Bran or corn muffins	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ 1 muffin	☐ 2 muffins	☐ 3 or more muffins
31.	Other muffins (blueberry, chocolate chip, cranberry)	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ 1 muffin	☐ 2 muffins	☐ 3 or more muffins
32.	Pancakes or waffles	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ 1	☐ 2	☐ 3 or more

CHECK

33. If you eat bread, pancakes or waffles, do you add:

	Always	Usually	Sometimes	Never
a) Diet margarine or light cream cheese				
b) Butter, margarine or regular cream cheese				
c) Low calorie mayonnaise or salad dressing				
d) Regular mayonnaise or salad dressing				
e) Calorie-reduced peanut butter				
f) Regular peanut butter				
g) Jelly, jam, honey, syrup				

34. If you eat muffins or crackers, do you add:

	Always	Usually	Sometimes	Never
a) Diet margarine or light cream cheese				
b) Butter, margarine or regular cream cheese				
c) Calorie-reduced peanut butter				
d) Regular peanut butter				
e) Jelly, jam, honey, syrup				

- **Meat, Fish, Poultry and Alternates**

		Do you eat or drink this at least once a month?	**How many times per day or week or month?**	**about how much do you have each time?**		
35.	Roasted, stewed or barbecued beef and steak	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 4 ounces	☐ more than 4 ounces	☐ less than 4 ounces
36.	Roasted, stewed or barbecued pork and pork chops	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 4 ounces	☐ more than 4 ounces	☐ less than 4 ounces
37.	Fried or breaded beef and steak	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 4 ounces	☐ more than 4 ounces	☐ less than 4 ounces
38.	Fried or breaded pork and pork chops	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 4 ounces	☐ more than 4 ounces	☐ less than 4 ounces
39.	Liver, any type	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 4 ounces	☐ more than 4 ounces	☐ less than 4 ounces
40.	Roasted, stewed or barbecued chicken, turkey or other poultry	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 4 ounces	☐ more than 4 ounces	☐ less than 4 ounces
41.	Fried chicken, nuggets, chicken sandwiches	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 2 pieces 6 nuggets 1 s'wich	☐ 4 pieces 9 nuggets 2 s'wiches	☐ more than 4 pieces/ 9 nuggets
42.	Fish: canned, fresh or frozen (tuna, salmon, sushi)	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 4 ounces	☐ more than 4 ounces	☐ less than 4 ounces
43.	Fried fish, fried fish sandwiches	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 2 pieces 1 s'wich	☐ 4 pieces 2 s'wiches	☐ more than 9 nuggets
44.	Hamburgers and cheeseburgers (count bun with Breads pg 4 #27)	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 4 ounces	☐ more than 4 ounces	☐ less than 4 ounces

		Do you eat or drink this at least once a month?	How many times per day or week or month?	about how much do you have each time?		
45.	Wieners, hot dogs (count bun with Breads pg 4 #27)	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 regular	☐ 1 large 2 regular	☐ more than 1 lg/2 reg.
46.	Bacon	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1-2 slices	☐ 3-4 slices	☐ 5 or more slices
47.	Sausages	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1-2 links	☐ 3-4 links	☐ 1-2 large sausages
48.	Sandwich meat: sliced roast beef	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1-2 slices	☐ 3-4 slices	☐ 5 or more slices
49.	Sandwich meat: sliced turkey, chicken, bologna, salami, ham	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1-2 slices	☐ 3-4 slices	☐ 5 or more slices
50.	Tofu, soy bean curd (tofu wieners, ground tofu)	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ more than ½ cup	☐ less than ½ cup

51. If you eat meat, do you:

	Always	Usually	Sometimes	Never
a) Add gravy or mayonnaise?				
b) Eat the fat or skin?				
c) Use tartar sauce or mayonnaise with fish?				

		Do you eat or drink this at least once a month?	How many times per day or week or month?	about how much do you have each time?		
	• **Mixed Dishes**					
52.	Meat pie	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1-2 slices	☐ 3-4 slices	☐ 5 or more slices
53.	Pizza with meat, Pizza Pops	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1-2 slices 1 Pizza Pop	☐ 3-4 slices 2 Piz. Pops	☐ 5 or more

		Do you eat or drink this at least once a month?	How many times per day or week or month?	about how much do you have each time?		
54.	Vegetable pizza without meat	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1-2 slices	☐ 3-4 slices	☐ 5 or more
55.	Spaghetti, lasagna or other pasta with meat sauce	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 cup	☐ more than 1 cup	☐ less than 1 cup
56.	Chili with meat	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 cup	☐ more than 1 cup	☐ less than 1 cup
57.	Chili without meat	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 cup	☐ more than 1 cup	☐ less than 1 cup
58.	Any other mixed dishes made with beef or ground beef	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 cup	☐ more than 1 cup	☐ less than 1 cup
59.	Any other mixed dishes made with chicken or fish	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 cup	☐ more than 1 cup	☐ less than 1 cup
60.	Macaroni and cheese or other pasta with cheese	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 cup	☐ more than 1 cup	☐ less than 1 cup
61.	Any other pasta or noodles without meat or cheese	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 cup	☐ more than 1 cup	☐ less than 1 cup
62.	Rice, any type	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 cup	☐ more than 1 cup	☐ less than 1 cup
	• **Soups**					
63.	All cream-type soup with or without meat	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 cup	☐ more than 1 cup	☐ less than 1 cup
64.	Soup with meat (beef vegetable, chicken noodle)	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 cup	☐ more than 1 cup	☐ less than 1 cup

		Do you eat or drink this at least once a month?	**How many times per day or week or month?**	**about how much do you have each time?**		
65.	Vegetable or noodle-type soup without meat (minestrone, broth)	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 cup	☐ more than 1 cup	☐ less than 1 cup
66.	Soup without meat but with lentils, beans or peas	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 cup	☐ more than 1 cup	☐ less than 1 cup
	• **Vegetables**					
67.	Broccoli	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ more than ½ cup	☐ less than ½ cup
68.	Carrots	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ more than ½ cup	☐ less than ½ cup
69.	Corn	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ½ cup small cob	☐ more than ½ cup	☐ less than ½ cup
70.	Green peas	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ more than ½ cup	☐ less than ½ cup
71.	Brussels sprouts	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ more than ½ cup	☐ less than ½ cup
72.	Greens (spinach, kale, bok choy, leeks)	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ more than ½ cup	☐ less than ½ cup
73.	Green beans, string beans, yellow beans	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ more than ½ cup	☐ less than ½ cup
74.	Other beans, peas, lentils (lima, navy, kidney, baked beans, pork & beans)	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ more than ½ cup	☐ less than ½ cup

		Do you eat or drink this at least once a month?	How many times per day or week or month?	about how much do you have each time?		
75.	Potatoes (baked, boiled, mashed, potato salad)	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 cup	☐ more than 1 cup	☐ less than 1 cup
76.	French fries, home fries pan fried potatoes, hash browns	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ small ½ cup	☐ medium 1 cup	☐ large 1½ cups
77.	Squash, all types	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ more than ½ cup	☐ less than ½ cup
78.	Salad - combination lettuce and tomato	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 cup	☐ more than 1 cup	☐ less than 1 cup
79.	Salad - spinach or bean	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 cup	☐ more than 1 cup	☐ less than 1 cup
80.	Any other salads such as coleslaw, carrot	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 cup	☐ more than 1 cup	☐ less than 1 cup
81.	Any other vegetables such as cabbage, asparagus	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ more than ½ cup	☐ less than ½ cup

CIRCLE ONE OR

CHECK

82.

	Always	Usually	Sometimes	Never
a) If you eat **potatoes or rice**, do you add:				
diet margarine, defatted gravy, diet sour cream				
butter, margarine, gravy or regular sour cream				
b) If you eat **vegetables**, do you add:				
diet margarine, low fat cheese or sauces				
butter, margarine, regular cheese or other sauces				
low fat vegetable dip or low fat salad dressing				
vegetable dip or dressing (ranch, blue cheese)				
c) If you eat **salads**, do you add:				
diet, low fat, low calorie mayo or salad dressing				
regular mayonnaise or dressing				

		Do you eat or drink this at least once a month?	**How many times per day or week or month?**	**about how much do you have each time?**		
	• **Fruit**					
83.	Apples, applesauce	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 apple ½ cup	☐ 2 apples 1 cup	☐ more than 2 apples/2 cups
84.	Bananas	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1	☐ 2	☐ 3 or more
85.	Oranges, grapefruit	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 orange ½ g'fruit	☐ 2 oranges 1 g'fruit	☐ more than 2 oranges/ 1 g'fruit
86.	Pears, peaches nectarines, grapes, plums	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 fruit ½ cup	☐ 2 fruit 1 cup	☐ more than 2 fruit/1 cup
87.	Raisins, prunes, other dried fruits	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ 1 cup	☐ more than 1 cup
88.	Cantaloupe	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ less than ¼ melon	☐ ¼ melon	☐ more than ¼ melon
89.	Any other fruit (berries, fruit cocktail, fruit salad)	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 fruit ½ cup	☐ 2 fruit 1 cup	☐ more than 2 fruit/1 cup
	• **Beverages**					
90.	Orange, grapefruit juice, 5-Alive	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ 1 cup	☐ more than 1 cup
91.	Apple, cranberry juice	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ 1 cup	☐ more than 1 cup
92.	Tomato, mixed vegetable juices	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ 1 cup	☐ more than 1 cup

		Do you eat or drink this at least once a month?	**How many times per day or week or month?**	**about how much do you have each time?**		
93.	Fruit drinks such as Kool-Aid, Tang, Fruitopia	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ 1 cup	☐ more than 1 cup
94.	Diet soft drinks	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ small or 1 can	☐ medium	☐ large
95.	Regular soft drinks	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ small or 1 can	☐ medium	☐ large
96.	Beer, wine or liquor	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 glass wine or 1 can beer or 1 oz liquor	☐ more than 1 gl. wine or 1 beer or 1 oz liq.	☐ less than 1 gl. wine or 1 beer or 1 oz liq.

REMEMBER, YOUR ANSWERS WILL BE KEPT STRICTLY CONFIDENTIAL.
YOUR NAME IS NOT ATTACHED TO ANY OF THESE SHEETS.

97.	Coffee	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 cup	☐ 2 cups	☐ 3 or more cups
98.	Cappuccino, espresso latté	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 cup	☐ 2 cups	☐ 3 or more cups
99.	Tea	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 1 cup	☐ 2 cups	☐ 3 or more cups

100. If you drink coffee cappuccino, espresso or latté:

a) Do you usually add sugar? ☐ Yes ☐ No
b) Do you usually add artificial sweetener? ☐ Yes ☐ No

c) Which one of the following do you usually use in your coffee? (circle one)

Cream or Whole Milk 2% Milk 1% Milk Skim Milk None

d) Do you usually drink coffee with meals? ☐ Yes ☐ No

101. If you drink tea:

a) Do you usually add sugar? ☐ Yes ☐ No

b) Do you usually add artificial sweetener? ☐ Yes ☐ No

c) Which one of the following do you usually use in your tea? (circle one)

Cream or Whole Milk   2% Milk   1% Milk   Skim Milk   None

d) Do you usually drink tea with meals? ☐ Yes ☐ No

		Do you eat or drink this at least once a month?	**How many times per day or week or month?**		**about how much do you have each time?**		
	• **Desserts and Snacks**						
102.	Ice cream, ice milk sherbet, frozen yogurt	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ 1 scoop	☐ 2 scoops	☐ 3 or more scoops
103.	Pudding	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ ½ cup (1 pudd cup)	☐ more than ½ cup (2 pudd cups)	☐ less than ½ cup
104.	Cake	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ 1 slice	☐ 2 slices	☐ 3 or more slices
105.	Pie	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ 1 slice	☐ 2 slices	☐ 3 or more slices
106.	Cookies	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ 1-5	☐ 6-10	☐ more than 10
107.	Donuts, danish, croissant	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ 1	☐ 2	☐ 3 or more
108.	Pop Tarts, Toaster Strudel, Wagon Wheels	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ 1	☐ 2	☐ 3 or more
109.	Granola bars	☐ Yes → ☐ No	____	☐ Day ☐ Week ☐ Month	☐ 1 bar	☐ 2 bars	☐ 3 or more

		Do you eat or drink this at least once a month?	How many times per day or week or month?	about how much do you have each time?		
110.	Chocolate bars	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ regular bar	☐ large bar	☐ 2 bars
111.	Potato chips, nacho chips, cheezies	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ small bag	☐ more than small bag	☐ less than small bag
112.	Popcorn	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 2 cups	☐ more than 2 cups	☐ less than 2 cups
113.	Pretzels	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ 2 cups	☐ more than 2 cups	☐ less than 2 cups
114.	Peanuts, other nuts and seeds, trail mix	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month	☐ ½ cup	☐ more than ½ cup	☐ less than ½ cup

- **Vitamin/Mineral Supplements**

		Do you use at least once a month?	How many capsules or tablets per day or week or month?
115.	Vitamin/mineral supplements	☐ Yes → ☐ No	____ ☐ Day ☐ Week ☐ Month

If you know the name of the product, please write it here________________________________

THE END

THANK YOU FOR TAKING THE TIME TO COMPLETE THIS QUESTIONNAIRE

APPENDIX H: General Health Information Questionnaire

ID #

GENERAL HEALTH INFORMATION QUESTIONNAIRE

Some of the questions are personal, but very important to our study. Please answer all questions as accurately as possible.

YOUR ANSWERS WILL BE KEPT *STRICTLY CONFIDENTIAL*.
YOUR NAME IS NOT ATTACHED TO ANY OF THESE SHEETS.

Please check (√)one:

Male: _____ Female: _____

Date of Birth: _____ (day) _____ (month) _____ (year)

A. Diet and Exercise Habits

Please check (√) one

	Yes	No	Sometimes
1. Do you think that you eat a balanced diet? ie. foods from all four groups daily: • vegetables and fruit • milk products • meat and alternatives • grain products	___	___	___
2. Are you eating **less** than usual to try to lose or maintain weight?	___	___	___
3. Are you eating **more** than usual to try to gain weight?	___	___	___
4. Do you classify yourself as a vegetarian?	___	___	___
If yes ⇒ a) Do you drink milk and/or eat other dairy products?	___	___	___
b) Do you eat fish or seafood?	___	___	___
c) Do you eat eggs?	___	___	___
d) Do you eat chicken or poultry?	___	___	___
e) Do you eat beef or other red meats? (hamburger, roast beef, steak)	___	___	___
5. Do you usually eat 3 meals/day? (not including snacks)	___	___	___

If no⇒ a) how many meals do you usually eat per day? _____ meals/day

ID #

Diet and Exercise Habits (cont'd) **Please check (√) one**

	Yes	No	Sometimes
6. Do you usually have snacks during the day?	___	___	___
7. Do you skip meals?	___	___	___

If yes⇒ a) On average, how many breakfast meals do you skip **per week**? ____ breakfast/week

b) On average, how many lunch meals do you skip **per week**? ____ lunch/week

c) On average, how many supper meals do you do skip **per week**? ____ supper/week

8. On average, how many meals **per week** do you eat at restaurants, cafeterias, or from vending machines? ______ meals/week

	Yes	No
9. Do you usually take a vitamin/mineral pill daily?	___	___

	Yes	No	Don't know
If yes ⇒ a) does it contain iron?	___	___	___

	Yes	No	Sometimes
10. On average, do you exercise 5 times/week or more?	___	___	___

If yes ⇒ a) please complete the table below.

Type of Activity	How long is each session?	At what intensity? (low, mod., heavy)	Number of times/week?

ID #

REMINDER:

ALL OF THIS INFORMATION IS *STRICTLY CONFIDENTIAL*

B. Medical Information

Please check (√) one

	Yes	No
1. In the last **6 months**, have you had an injury that resulted in a large amount of blood loss?	___	___
2. In the last **6 months**, have you had any stomach or intestinal problems that resulted in a large amount of blood loss?	___	___
3. In the last **6 months**, have you had any infections? (flu, colds, ear infections)	___	___
If yes ⇒ a) how many?	___ /6 months	
4. Do you have any long term illnesses? (diabetes, kidney disorders, thyroid disorders, asthma)	___	___

If yes ⇒ a) please state which illness(es)

____

____

5. Have you ever been diagnosed as anemic (low blood iron)?	___	___

If yes ⇒ Condition:_________________________

Time Period:_________________________

Treatment:_________________________

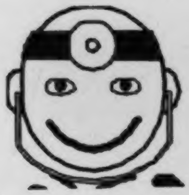
6. Have you donated blood in the last **2 months**?	___	___

If yes ⇒ Date: _____ _____ _____
day month year

7. Are you taking any medications, or have you taken any in the past 6 months? (this includes the birth control pill)	___	___

If yes ⇒ a) please state **which ones** and **for how long**.

Type of medication	For how long?



ID #

Medical Information (cont'd)

	Please check (√) one	
	Yes	**No**
[Questions 8 & 9 are for FEMALES ONLY]		
8. Have you started having menstrual periods?	___	___
If yes ⇒ a) is the flow **light**, **medium**, or **heavy**? (circle one)		
If yes ⇒ b) do you have regular menstrual periods? (approximately once per month)	___	___
9. When did you get your first menstrual period?	____ (Age)	

C. General Health and Well-Being

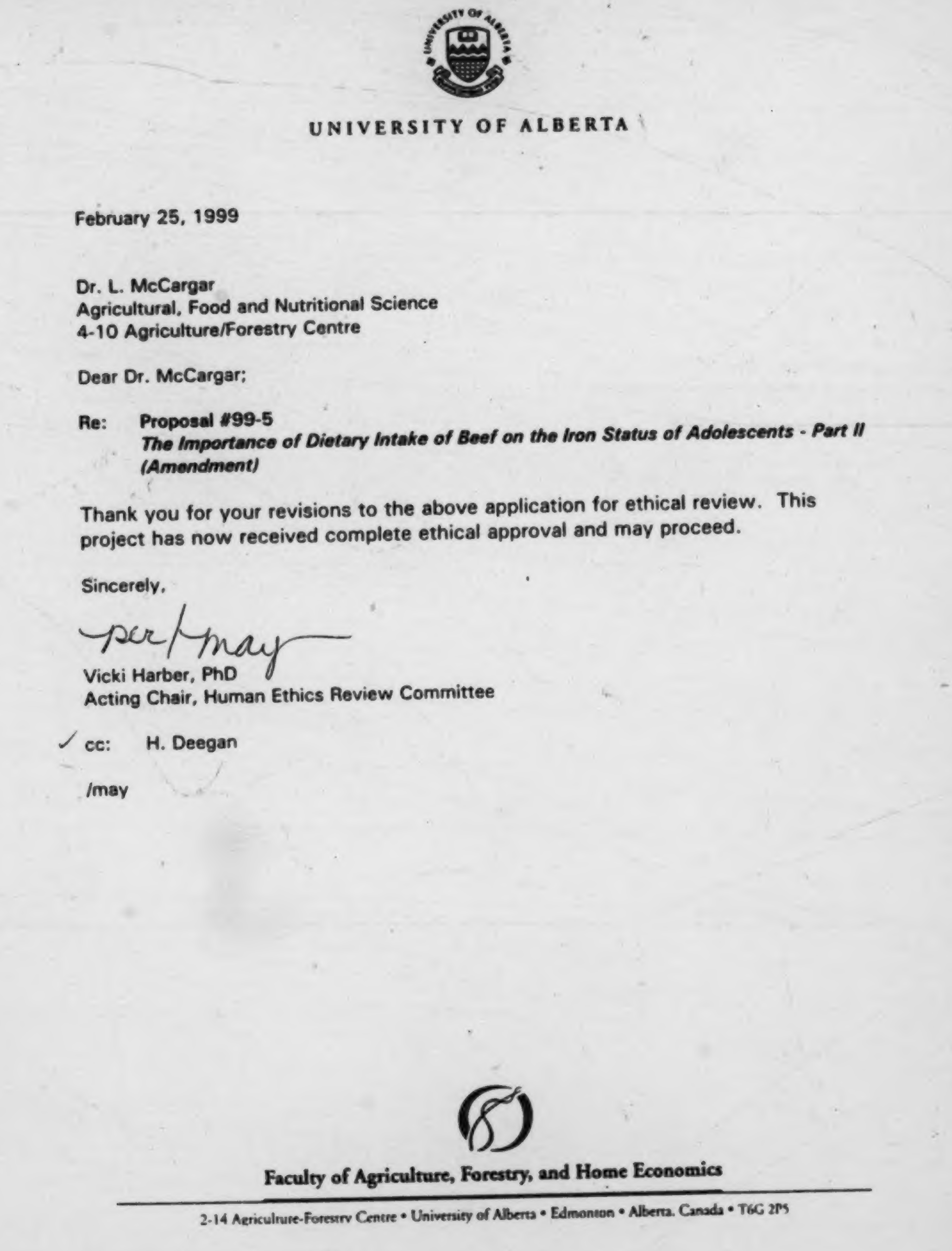
	Please check (√) one	
	Yes	**No**
1. Do you smoke cigarettes?	___	___
If yes ⇒ a) what is the average number of cigarettes you smoke **per week**?	____ /week	
2. Are you often tired, even after a good amount of sleep?	___	___
3. Do you feel that you concentrate well at school?	___	___

4. How would you rate your overall health? (circle one)

1 (very poor)
2
3 (poor)
4
5 (satisfactory)
6 (good)
7
8 (very good)
9
10 (excellent)

Thank you for completing this questionnaire.

APPENDIX I: Approval-Human Ethics Review Committee-Calibration Study



UNIVERSITY OF ALBERTA

February 25, 1999

Dr. L. McCargar
Agricultural, Food and Nutritional Science
4-10 Agriculture/Forestry Centre

Dear Dr. McCargar;

Re: **Proposal #99-5**
The Importance of Dietary Intake of Beef on the Iron Status of Adolescents - Part II (Amendment)

Thank you for your revisions to the above application for ethical review. This project has now received complete ethical approval and may proceed.

Sincerely,

per/may

Vicki Harber, PhD
Acting Chair, Human Ethics Review Committee

✓ cc: H. Deegan

/may

Faculty of Agriculture, Forestry, and Home Economics

2-14 Agriculture-Forestry Centre • University of Alberta • Edmonton • Alberta, Canada • T6G 2P5

APPENDIX J: Consent Form – Calibration Study

UNIVERSITY OF ALBERTA

INFORMATION SHEET

TITLE OF RESEARCH PROJECT: **The Importance of Dietary Intake of Beef In the Iron Status of Adolescents - Part II**

INVESTIGATORS:	Linda McCargar PhD RD	492-9287
	Heather Deegan RD MSc Candidate	492-4267

Background: Your daughter/son is already involved in a large research study (the Iron Study) assessing the iron status and dietary intake patterns of Edmonton area adolescents. The research team would like to do a follow-up study with a selected group of research participants and compare their dietary intake information to the larger study group. Obtaining more information on dietary intake will provide us with a more precise estimate of the actual nutrient consumption of adolescents.

Purpose: The dietary assessment tool being used in the current study will be help track actual foods and beverages consumed over a three day period. This information will be compared to estimated dietary intake information obtained earlier in the Iron Study and will help determine the accuracy and validity of the Food Frequency Questionnaire as a dietary assessment tool.

Procedures: During class time, The Research Co-ordinator will instruct participants on how to accurately record their food and beverage intake. Students will be provided with a booklet to take home and record all foods they consume over a specified three day period. After the intake period, Food Intake Records will be collected by the Research Co-ordinator, analyzed for nutrient content and compared to information obtained with the Food Frequency Questionnaire. previously used in the Iron Study.

Possible Benefits/Risks: There are no risks involved for students participating in the study.

Confidentiality: Personal records relating to this study will be kept strictly confidential. All files will be stored in a locked file cabinet. Any report published as a result of this study will present group results only and no individual will be identified.

Compensation: An incentive for participation will be offered in the form of a draw for prizes.

Time Commitment: Dietary Intake Record instruction will take place during class time and will take approximately 10 minutes. Participants are expected to keep an accurate record of their food and beverage intake on their own time. This will take approximately 60 minutes (20 minutes a day x 3 days).

Results: Each participant will have the opportunity to obtain their dietary intake results.

Withdrawal From Study: You are free to withdraw from this research study at any time.

Any Questions? Please contact:

Heather Deegan RD, MSc Candidate
Research Co-ordinator
Department of Agricultural, Food and
Nutritional Science Phone: 492-4267

-OR-

Linda McCargar PhD RD
Associate Professor
Department of Agricultural, Food and
Nutritional Science Phone: 492-9287

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...it makes

Department of Agricultural, Food and Nutritional Science
Faculty of Agriculture, Forestry, and Home Economics

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Consent Form

I acknowledge that the research procedures described on the Information Section are clear to me. Any questions I may have had were answered to my satisfaction. In addition, I know that I may contact the persons designated on this form if I have further questions either now or in the future. I have been assured that personal records relating to this study will be kept confidential. I understand that I am free to withdraw from the study at any time without jeopardy to myself.

(Name of Participant)

(Signature of Participant) *must be obtained first

(Name of parent or legal guardian of participants under the age of 18)

(Signature of parent or legal guardian of participants under the age of 18)

(Date)

(Name of Witness)

(Signature of Witness)

(Signature of Investigator)

Individuals who may be contacted about the research are:

Heather Deegan RD MSc Candidate
Research Co-ordinator
Agricultural, Food & Nutritional Science
Phone: 492-4267

-OR-

Linda McCargar PhD RD
Associate Professor
Agricultural, Food & Nutritional Science
Phone: 492-9287

☐ Yes, I would like my 3-Day nutrient analysis mailed to my home once the research is complete.

Address:

APPENDIX K: Dietary Intake Record

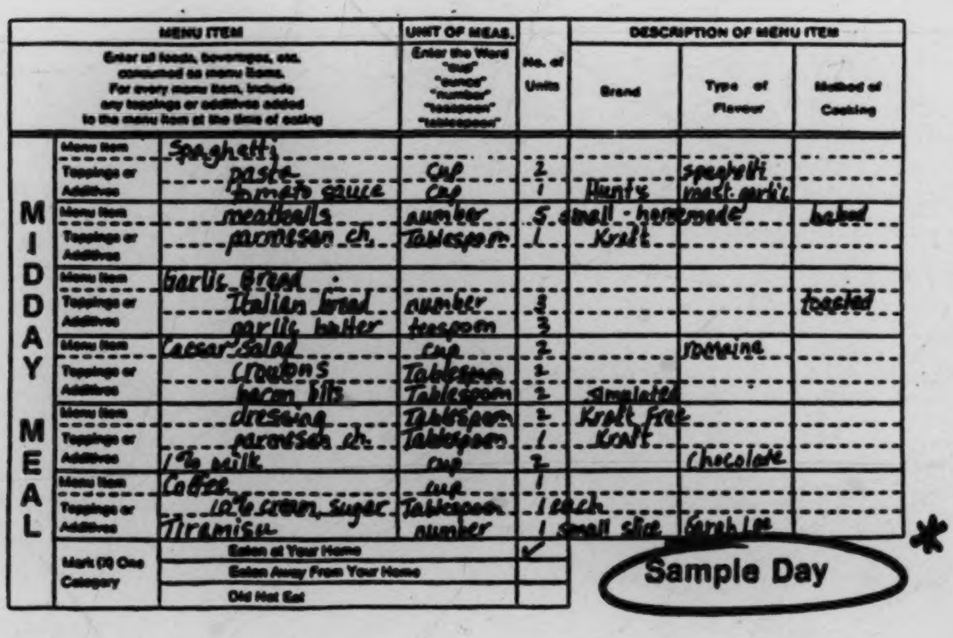
DIETARY INTAKE RECORD

Name: ____________________

Phone Number: ________________

Date of Birth: ___ ___ ___
(Day) (Month) (Year)

Record Dates: ___ ___ ___ ___ ___ ___
(Day) (Month) (Day) (Month) (Day) (Month)
Day One Day Two Day Three



MENU ITEM		Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM: Brand	Type or Flavour	Method of Cooking
MIDDAY MEAL	Menu Item	Spaghetti					
	Toppings or Additives	pasta	cup	2		spaghetti	
		tomato sauce	cup	1	Hunts	roast garlic	
	Menu Item	meatballs	number	5	small - homemade		baked
	Toppings or Additives	parmesan ch.	Tablespoon	1	Kraft		
	Menu Item	Garlic Bread					
	Toppings or Additives	Italian bread	number	2			toasted
		garlic butter	teaspoon	3			
	Menu Item	Caesar Salad	cup	2		romaine	
	Toppings or Additives	croutons	Tablespoon	2			
		bacon bits	Tablespoon	2	simulated		
	Menu Item	dressing	Tablespoon	2	Kraft free		
	Toppings or Additives	parmesan ch.	Tablespoon	1	Kraft		
		1% milk	cup	2		Chocolate	
	Menu Item	Coffee	cup	1			
	Toppings or Additives	10% cream, sugar	Tablespoon	1 each			
		Tiramisu	number	1	small slice	Sarah Lee	
	Mark (X) One Category	Eaten at Your Home		✓			
		Eaten Away From Your Home					
		Did Not Eat					

* Sample Day

MENU ITEM			UNIT OF MEAS.	No. of Units	DESCRIPTION OF MENU ITEM		
Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating			Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"		Brand	Type of Flavour	Method of Cooking
MIDDAY MEAL	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Mark (X) One Category	Eaten at Your Home					
		Eaten Away From Your Home					
		Did Not Eat					

Day Three

MENU ITEM			UNIT OF MEAS.	No. of Units	DESCRIPTION OF MENU ITEM		
Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating			Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"		Brand	Type of Flavour	Method of Cooking
AFTERNOON SNACK	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Mark (X) One Category	Eaten at Your Home					
		Eaten Away From Your Home					
		Did Not Eat					

Day One

MENU ITEM			UNIT OF MEAS.		DESCRIPTION OF MENU ITEM		
Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating			Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	Brand	Type of Flavour	Method of Cooking
EVENING MEAL	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Mark (X) One Category	Eaten at Your Home					
		Eaten Away From Your Home					
		Did Not Eat					

Day One

MENU ITEM			UNIT OF MEAS.		DESCRIPTION OF MENU ITEM		
Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating			Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	Brand	Type of Flavour	Method of Cooking
MIDMORNING SNACK	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Mark (X) One Category	Eaten at Your Home					
		Eaten Away from Home					
		Did Not Eat					

Day Three